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KLOSSIELLA INFECTION OF THE GUINEA PIG.

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(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

PLATES 63 TO 70.

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In studying the lesions produced by arsenical compounds in the kidneys of different species of animals, certain parasites were observed in the renal tubules of guinea pigs, which strikingly resembled the coccidium, *Klossiella muris*, observed and described by Smith and Johnson[1, 2] in the kidneys of the mouse. As we believed the guinea pig to be relatively free from spontaneous renal lesions, a search through the literature was made to ascertain if renal parasites of the guinea pig had been previously described. At the same time the study of a large number of guinea pig kidneys was undertaken, in order to determine the relative frequency of this parasite as well as to obtain information concerning the lesions which tend to be associated with it.

Seidelin[3] in 1914 describes a parasite which occurred in the kidneys of two guinea pigs from Nigeria, and which bears such strong resemblance to *Klossiella muris* in some of its stages that he considers that both parasites "must be regarded as belonging to one and the same genus, whilst the question of their specific identity or otherwise cannot at the present time be finally decided." He further states that the parasite does not appear to be a common one in West African guinea pigs, as he found only one case among about twenty guinea pigs of the Yaba series. Later he found identical parasites in sections of a kidney of another guinea pig from the same locality. He found no similar instance in guinea pigs procured in Liverpool.

[1] Smith, T., *Jour. Comp. Med. and Surg.*, 1889, x, 211.
[2] Smith, T., and Johnson, H. P., *Jour. Exper. Med.*, 1914, vi, 303.
[3] Seidelin, H., *Ann. Trop. Med. and Parasit.*, 1914–15, viii, 553.

Seidelin describes some of the stages of the parasite detected by him and suggests a probable life cycle. There is undoubtedly a close resemblance between this parasite and *Klossiella muris*, and again between both these parasites and the one about to be described. On the other hand, the question of the identity of Seidelin's parasite and the one we have observed will be discussed later on in this paper. There are certainly points of similarity between the two, as well as some points of difference.

It may be stated here that we have found the infection to be by no means uncommon in kidneys of guinea pigs. In sixty guinea pigs examined for renal parasites, twelve were found to be infected. In other words, in our experiments with guinea pigs we found that we must reckon upon at least 20 per cent of the animals from our sources being infected with this renal parasite.

The material examined consisted of kidney sections of sixty adult guinea pigs from two sources, one in Philadelphia, the other in New Brunswick, New Jersey. These pigs were either perfectly normal animals or were animals which had been used for toxicological experiments with various arsenical compounds and had survived the use of the drug not more than 24 hours. This arbitrary time limit was set in the choice of material in order that any chronic pathological process observed might not be confused with the drug action, since within this time (24 hours) no extensive proliferative change in the kidney can be attributed to the action of these compounds. The kidney tissue was fixed in Zenker's fluid and imbedded in paraffin. Sections were stained with hematoxylin and eosin, methylene blue and eosin, and Giemsa's stain. In addition, frozen sections were made from tissue fixed in 10 per cent formalin and stained with hematoxylin and eosin.

Description of the Parasite.

Various stages of the parasite have been seen in the kidneys of guinea pigs and in order to facilitate an interpretation of its probable life cycle, a certain sequence of description will be observed, beginning with the stage or form most frequently encountered.

Sporoblast Cycle.—The form most frequently seen is a small round or ovoid body measuring 7 to 8 μ in diameter. It stains a very light

bluish pink with hematoxylin and eosin, and contains numerous small dark blue masses of chromatin scattered through the cytoplasm in no definite arrangement (Figs. 1 and 2). The cytoplasm is somewhat granular and slightly, if at all refractile. The chromatin is of two sizes, larger irregular masses and tiny pin points, both taking a dark blue stain with hematoxylin and methylene blue. Usually several of these parasites are found free in the lumen of the renal tubules and sometimes the lumen appears to be completely blocked with them. They are most frequently seen in the straight tubules of the inner half of the cortex, but are also found somewhat less numerously in the convoluted tubules and in the tubules of the upper medulla. Occasionally one or two may be found in the capsular space of the glomerulus. This stage of the parasite is apparently very similar to the so called "daughter sporoblast" stage of *Klossiella muris* described by Smith and Johnson.

In certain sections it has been possible to make out a confining cell membrane surrounding several of these bodies, as in Fig. 2. The membrane is all that remains of the epithelial cell that originally contained the developing parasites. As they grow and increase in size the host cell must necessarily enlarge until it becomes flask-shaped and bulges out into the lumen of the tubule. The parasites occupy the broad distal portion of the containing cell and the nucleus is usually crowded inwards and often to one corner of the cell. It frequently appears flattened, compressed, or shrunken, and eventually it disappears, apparently just preceding or about the time of rupture of the cell. The cytoplasm of the host cell gradually becomes more and more scanty and granular and finally all that remains of the cell is its enormously distended cell wall, which is ruptured by the escaping parasites. It is somewhat difficult to determine just how many parasites develop in the epithelial cell, as we have had no opportunity of examining any material except cross sections of kidney tissue. However, there are probably at least ten to twelve.

Each of these parasites next undergoes a division or segmentation into eight to twelve bodies (Fig. 3). These bodies are falciform or banana-shaped and are extremely small, measuring 1 by 4 to 5 μ, and are surrounded by a distinct membrane. Their cytoplasm is clear and non-granular, pink (hematoxylin and eosin, and Giemsa's

stain), and each body contains a dark blue dot of chromatin. We believe that these tiny fusiform bodies are similar to the form designated as the sporozoites of *Klossiella muris*. The further history of these bodies is entirely conjectural and will be referred to later in the discussion.

Not uncommonly one will find in heavily infected kidneys a form (Fig. 4) which is evidently the precursor of the two stages just described. This form is comparatively large, and may measure 17 by 22 μ; it has the same appearance and staining characteristics as the smaller more usual forms first described (Figs. 1 and 2). A breaking up of this large mass into several constituent smaller bodies results in a picture similar to Fig. 2. In studying various infected kidneys, we have seen several microscopic fields in which there were all gradations of this division (Fig. 6), so that we are inclined to regard this large parasitic mass as corresponding to the so called "mother sporoblast" stage of *Klossiella muris*.

Ring Form.—In several of the sections of heavily infected kidneys we have seen a stage of the parasite which we have called the "ring," or "annular" form (Figs. 7 and 8). It is always within an epithelial cell and the cell itself is enlarged and may protrude into the lumen of the tubule to the extent of nearly occluding it, as in Fig. 8. This form measures 18 to 20 μ in diameter and consists of a series of twelve to eighteen definite divisions or segments arranged in a circle or ring. Each division is extremely small, ovoid in shape, and measures 3 by 5 to 7 μ. They are smaller than the more frequently encountered forms seen in Figs. 1, 2, 3, and 4 (the daughter sporoblasts), and in appearance are totally different. The cytoplasm is refractile and stains practically not at all, or at most a very pale pink with hematoxylin and eosin, and Giemsa's stain. Each segment contains one or two chromatin dots. In some instances the two chromatin dots appear to be fusing together; in others they are distinctly separate from one another. In other instances the one chromatin dot is elliptical in shape, and it is possible that the finding of two chromatin dots is due to the level of the section. In the center of the ring form is a small amount of a granular refractile pale pink staining material which is apparently a residuum of the cytoplasm of the surrounding ring segments (Figs. 7 and 8).

In Fig. 8 there is a typical ring body and another intracellular form which we think is also a ring body seen from the outside. In other words, a ring body is a section through such a hollow sphere as is seen in Figs. 9 and 10. Here there are eighteen small segments or divisions, each of which is similar in appearance and staining reactions to the segments of the ring form.

The ring segments are not only considerably larger than the final divisions of the sporoblast cycle, the sporozoites (Fig. 5), but are also of a totally different shape and appearance. Moreover, the immediate precursor stage, the ring form, is still enclosed in an epithelial cell, while the precursor stage of the falciform bodies shown in Fig. 5 is not necessarily intracellular. Indeed, when the division into the tiny falciform bodies or sporozoites occurs, the daughter sporoblasts are probably always extracellular and free in the lumen of the tubule. In addition, the sporozoites are contained in a very distinct spore, having a definite membrane, while there is no such structure surrounding the segments of the ring form. When the ring divides, the resulting segments are all apparently set free in the remains of the epithelial host cell and eventually in the lumen of the tubule when this cell ruptures. The ring segments seem to be identical with still another stage of the parasite about to be described and may represent its earliest and youngest form.

Hyaline Forms.—Figs. 11 to 16 illustrate a single stage of the parasite which from its appearance and staining character is evidently one of the segments of the ring form. It is very small, measuring from 5 to 8 μ in length and 3 to 5 μ in breadth, and is oval or ovoid in shape. The cytoplasm is non-granular, extremely refractile, hyaline, and stains pink with hematoxylin and eosin, or Giemsa's stain. It contains a relatively large clear-cut mass of dark blue staining chromatin placed towards one end of the parasite. At the opposite pole a small clear non-staining area may be seen, as in Fig. 12. In the section from which Fig. 11 was taken this clear area is visible, but it is very small and barely shows in the photograph. This stage measures 3 by 5.5 to 7 μ. It may be extra- or intracellular, as shown in the illustrations. In Fig. 12 it apparently is about to enter an epithelial cell of a renal tubule. In Fig. 11 it is clearly intracellular and surrounded by a vacuole. The nucleus of the host

cell is immediately below the parasite. The epithelial cell opposite the parasite on the other side of the renal tubule contains an ovoid inclusion which is probably a similar parasitic body, although the mass of chromatin is not seen in the section. The cytoplasm of the two cellular inclusions is identical.

In Fig. 13 another intracellular parasite is seen and here the chromatin has divided into two distinct parts. The parasite itself measures 5 by 7 μ. The host cell has become enormously enlarged and protrudes into the lumen of the tubule. Fig. 14 shows a further division of the chromatin into four distinct segments. This parasite is also intracellular, but in order to bring the chromatin into focus for the photograph, the epithelial cell outlines are not seen. Just below this parasite is another smaller intracellular form similar to those shown in Figs. 11 and 12, with only one chromatin mass.

Schizogonic Cycle.—Fig. 17 illustrates a spherical form which we are inclined to believe represents the schizogony of the parasite. It it comparatively large—measuring 22 μ in diameter, and is composed of a large number (thirty to forty) of tiny fusiform bodies or merozoites enclosed in a rather indistinct and apparently very thin membrane. It does not appear to be enclosed in an epithelial cell, but is extracellular and free in the lumen of the renal tubule. The merozoites in longitudinal section measure 1.5 by 7 to 8 μ. Their cytoplasm stains pink with hematoxylin and eosin, and each contains a tiny dot of chromatin. These small falciform bodies are extremely similar to the sporozoites described above. The general appearance of this large form suggests its similarity, if not identity with the glomerular body of *Klossiella muris*, which Smith and Johnson interpret as the schizogony of the mouse parasite. We have not found this form in the capsular space of the glomeruli but in the convoluted tubules of the first order. It is not a common stage and we have found it in only one kidney.

Pathological Changes in the Kidneys of Guinea Pigs Associated with the Parasitic Infection.

The kidneys of guinea pigs infected with the parasite show certain pathological changes of a chronic nature which we are inclined to attribute to the presence of the organism. They have been found

in all cases in which the parasite has been seen, and in several instances where these lesions were observed the infection was very light, and the parasite was found only after a thorough search.

The lesions which we think are caused by the parasite consist in an irregular accumulation of fibroblasts and small round cells about the base of some of the glomeruli. Both the distribution and arrangement of this infiltration are very irregular (Figs. 18 to 21). In a single microscopic field one or two glomeruli may be affected in this manner, while the adjacent glomeruli are normal in appearance. Moreover, the extent of the infiltration varies considerably. Some glomeruli have only a slight accumulation of round cells about their base, others are almost obliterated, as in Figs. 18, 20, and 21. Usually there seem to be relatively more cells of the small round mononuclear type than fibroblasts. The fibroblasts themselves are apparently not young cells.

These cellular accumulations seem to be fairly well confined to the immediate vicinity of the glomeruli. In certain instances, however, the round cells and fibroblasts extend outward to a limited degree into the labyrinthine tissue and along the medullary rays between the tubules, but in these cases the connection between this extension and the accumulation of similar cells about the neighboring glomeruli can be easily traced.

There seems to be no reaction of the kidney tissue in the immediate vicinity of the parasite itself, that is, in the inner half of the cortex where we have found the parasite to be most numerous. Here one may see half a dozen consecutive tubules filled with parasites and eight or ten epithelial cells containing ring forms, yet there is apparently no abnormality in the immediate interstitial connective tissue. The glomeruli, however, just above these infected tubules show a more or less extensive infiltration of round cells and fibroblasts. The portion of medulla just below the infected tubule shows no appreciable change. No gross changes in the kidneys referable to the renal parasite have been noted.

DISCUSSION.

The description of the various stages of the parasite found in the kidney of the guinea pig has been arranged so as to relate, tentatively at least, those forms which seem to belong to the same cycles

of development. Certainly two different cycles of development have been observed. The exact interpretation of the cycles must be more or less hypothetical, since there is an obvious lack of knowledge of all the stages in the evolution of the parasite.

The first cycle, described under the sporozoites is similar to the so called sporoblast stage of *Klossiella muris* and is evidently similar to the stage described by Seidelin. However, we have never seen more than twelve daughter sporoblasts, and usually only eight to ten resulting from the first division of the mother or pan sporoblast form, while Seidelin gives sixteen to twenty as the probable number. This discrepancy, if the two parasites are identical, can probably only be settled by the study of fresh material or an extensive series of sections. In the second division, into sporozoites, we have never observed more than twelve, each sporozoite measuring 1 by 4 to 5 μ. Seidelin describes thirty sporozoites, each measuring 1.5 by 8 μ. If this difference in the number of sporozoites continues to hold after the study of fresh tissue, we shall be inclined to believe that the two parasites belong to different species.

That this cycle of the parasitic development represents the sporoblast phase is strongly suggested by the fact that the sporozoites are confined in what is apparently a typical spore. We have never seen these spore-like bodies breaking up in the lumen of the kidney tubules, and it is probable that the spores containing the sporozoites are excreted in the urine, which is afterwards swallowed by the same or other guinea pigs, and that the spore membrane is digested away by the gastric juice, thus freeing the sporozoites. The extremely small size and fusiform shape of these tiny bodies would doubtless enable them to pass through the gastric or intestinal mucosa into the blood stream and so into the kidney. On the other hand, we have never seen any sporozoites in the glomerular tuft or capsular space.

The ring forms are not numerous, but they are easily found in heavily infected kidneys. They differ markedly from any stages of the sporoblast cycle and do not appear to be an integral part of it. It is easy to differentiate between a mother sporoblast, for instance, and a ring form seen from the outside, or cut on a tangent, so that the annular appearance is not seen. Moreover, the resulting division or segments of the two forms are apparently very dissimilar. The

sporozoites are slender fusiform bodies, 1 by 4 μ, with a tiny dot of chromatin; the ring segments are oval or ovoid, 3 by 5 to 7 μ, with a relatively large mass of chromatin. The ring segments are apparently identical with the small hyaline forms seen free in the lumen of the renal tubules or in the epithelial cells, and there is nothing in the nature of a spore membrane about the dividing ring to prevent their escape into the tubules when the ring completely segments. The final interpretation is, of course, one of conjecture only, but the cycle is strongly suggestive of a sexual phase. The comparatively large number of ring segments or hyaline forms suggests further that they may be the microgametes, and that the ring form may be the microgametocyte. We have not seen any bodies which we could interpret as macrogametes, or any process of fertilization or conjugation, unless Fig. 16 represents this phase.

Smith and Johnson describe in the sporoblast stage of *Klossiella muris* a budding process in which the chromatin occupies the periphery of the budding masses. One might think that the ring form is simply a cross section of some of these buds of the mother sporoblast. But the ring form possesses not only a different type of protoplasm from the mother or daughter sporoblast, but in addition its segments are totally unlike either the daughter sporoblasts on the one hand or the sporozoites on the other.

The asexual or schizogonic cycle is apparently represented by the large extracellular segmenting form seen in Fig. 17. It resembles the glomerular body described by Smith and Johnson and interpreted by them as the schizogonic form. We have not seen it in the glomerular space, but only in the convoluted tubules and its scarcity may be tentatively explained on the assumption that such a stage is present only in early and light infections. Later, apparently, the sporoblast cycle may supersede the schizogony, for this cycle only has been detected in our specimens of heavily infected kidneys. Its extremely large size and the great number of its segments or divisions preclude its belonging to either the sporoblast or ring cycles. Moreover, its situation free in the convoluted tubules is one of the locations where one would expect to find the development of such a stage, if, as we have already suggested, the infecting sporozoites find their way into the kidney by the blood stream. The merozoites or

segments of this large body are similar to the sporozoites, the final divisions of the sporoblast cycle. The conspicuous difference between the two stages of segmentation, aside from the difference in their number, is that the sporozoites are enclosed in a definite spore membrane, while the existence of a membrane surrounding the merozoite is problematical. In the specimens we have seen there is a very indistinct membrane, which, as in Fig. 17, is apparently ruptured, allowing the escape of the merozoites into the renal tubules.

Seidelin found no glomerular bodies as described by Smith and Johnson for *Klossiella muris*, but he considers that some of his tubular forms appear identical with the glomerular forms depicted by them. Unfortunately, Seidelin gives no illustration of this particular tubular form, so that we are unable to compare satisfactorily the two apparently corresponding stages. Seidelin is inclined to the opinion that these tubular forms represent the schizogony. Smith and Johnson think that their glomerular body is the schizogony for *Klosiella muris* and that the tubular forms are stages in the sporoblast cycle.

The pathological changes in the kidney of guinea pigs which we associate with the presence of this parasite are slight but definite and consist of an irregular accumulation of round cells and fibroblasts about some of the glomeruli. There is but slight involvement of the labyrinthine tissue adjacent to the glomeruli and apparently none at all in the lower or inner half of the cortex where the majority of the parasites are found. This may be due to the fact that the infecting sporozoites enter the kidney by way of the glomerular capillaries and that here the most serious injury to the kidney occurs, with a subsequent infiltration of round cells and fibroblasts.

SUMMARY.

We have found in the kidneys of twelve supposedly normal guinea pigs, coming from Pennsylvania and New Jersey, a parasite that closely resembles in some of its phases *Klossiella muris*, described by Smith and Johnson, and the renal parasite of two West African guinea pigs, described by Seidelin.

The forms most commonly found by us and described as the sporoblast cycle, are evidently similar to those described by Smith

and Johnson and by Seidelin. There are certain discrepancies of measurement between the parasite described by Seidelin and the one here described, but the most important difference between the two is the different number of sporozoites resulting from a final division of the daughter sporoblasts. Seidelin has found thirty sporozoites; we have found from eight to twelve, while the usual number is eight. Further, we have found a ring form which is unlike any of the stages in either the sporoblast or schizogonic cycle, and which we interpret tentatively as the male element or microgamete. In addition, we have found a tubular form which resembles the glomerular body of *Klossiella muris* and which we think is the schizogonic phase of this parasite.

EXPLANATION OF PLATES.

The illustrations are all from untouched microphotographs except Fig. 8, which is a drawing of an actual microscopic field. All the specimens except Fig. 17 are from Zenker fixed tissue.

PLATE 63.

FIG. 1. Renal tubules showing a heavy parasitic infection in different stages, the majority being daughter sporoblasts. Hematoxylin and eosin. × 675.

FIG. 2. Eight daughter sporoblasts enclosed in an epithelial cell of a convoluted tubule. Hematoxylin and eosin. × 1,000.

FIG. 3. Three spores containing seven to nine sporozoites. Note the distinct spore membrane. Hematoxylin and eosin. × 1,000.

PLATE 64.

FIG. 4. Mother sporoblast enclosed in an epithelial cell. The cell outline is not distinct in the photograph. Giemsa's stain. × 1,000.

FIG. 5. Glomerulus containing a single parasite, probably a young mother sporoblast in the capsular space. Hematoxylin and eosin. × 1,000.

FIG. 6. Three greatly enlarged epithelial cells, two containing mother sporoblasts, the third in the center containing eleven daughter sporoblasts. Hematoxylin and eosin. × 1,000.

PLATE 65.

FIG. 7. Two ring forms in adjoining tubules, each enclosed in an enlarged epithelial cell. The ring to the right shows ten divisions. Hematoxylin and eosin. × 1,000.

FIG. 8. Two ring forms in distended epithelial cells; the lower form is seen from the outside. Hematoxylin and eosin. × 1,000.

PLATE 66.

FIG. 9. Two ring bodies and several daughter sporoblasts. The ring body on the left is a tangential section through the edge of a hollow sphere. Methylene blue and eosin. × 1,000.

FIG. 10. The same field as in Fig. 9, but at a lower level, showing that the so called ring body is a section through a hollow sphere. Methylene blue and eosin. × 1,000.

PLATE 67.

FIG. 11. Two intracellular hyaline forms, the one to the right showing chromatin. Hematoxylin and eosin. × 1,000.

FIG. 12. An extracellular spindle-shaped hyaline body showing the chromatin mass at one pole and a clear non-staining area at the opposite pole. One extremity of this hyaline form is in the protoplasm of an epithelial cell. Hematoxylin and eosin. × 1,000.

FIG. 13. An intracellular hyaline form in an enlarged epithelial cell. The chromatin has divided into two masses. The parasite is in a vacuole in the host cell. Hematoxylin and eosin. × 1,000.

FIG. 14. Two intracellular hyaline forms; the chromatin of the upper parasite has divided into four masses. Giemsa's stain. × 1,000.

PLATE 68.

FIG. 15. Two small intracellular hyaline forms. Giemsa's stain. × 1,000.

FIG. 16. Two intracellular hyaline bodies. The larger one shows two elongated chromatin masses. The smaller parasite to the left is at a level which shows no chromatin. Giemsa's stain. × 1,000.

FIG. 17. A schizogonic form showing thirty to forty merozoites. This body is free in the tubule and has no definite membrane surrounding it. Taken from a frozen section fixed in 10 per cent formalin. Hematoxylin and eosin. × 1,000.

PLATE 69.

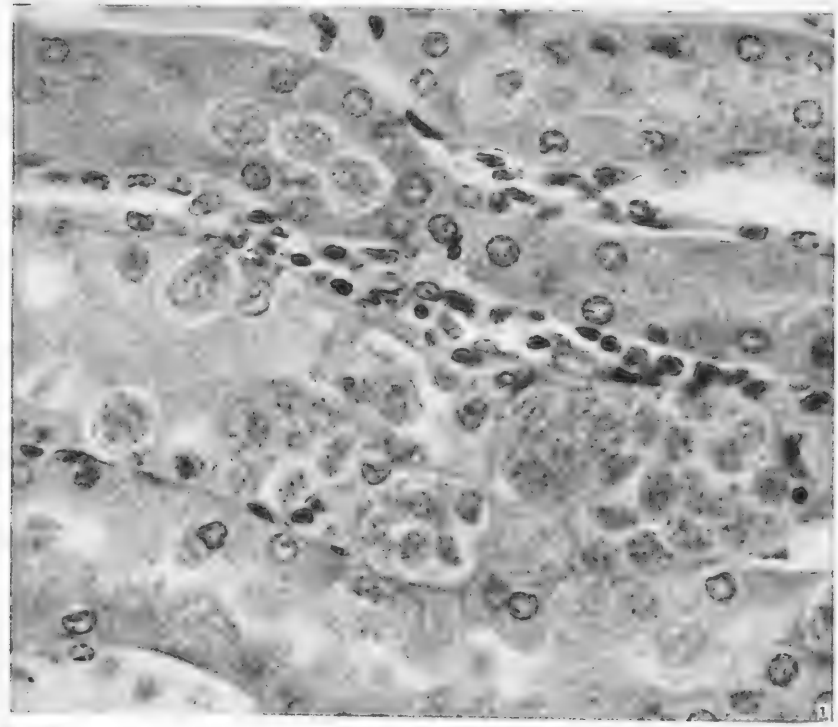
FIG. 18. Irregular cellular infiltrations, especially about the base of some of the glomeruli. Hematoxylin and eosin. × 125.

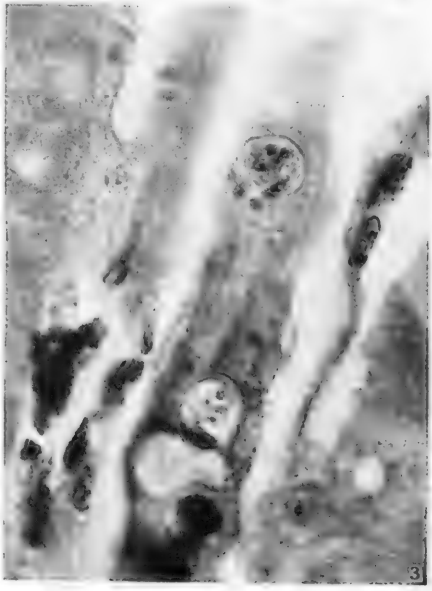
FIG. 19. Three glomeruli showing very slight cellular infiltration about the base. Two glomeruli show no such infiltration. Hematoxylin and eosin. × 210.

PLATE 70.

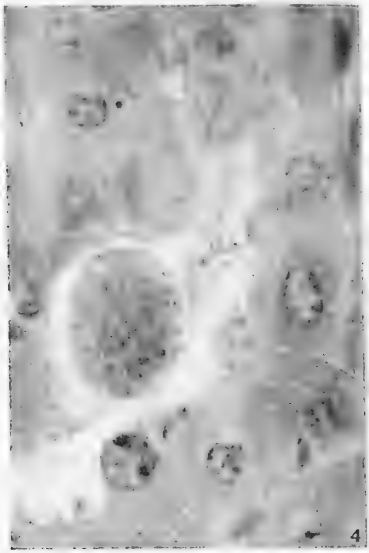
FIG. 20. The two lower glomeruli show a slight irregular accumulation of round cells and fibroblasts. The upper glomeruli are normal. Hematoxylin and eosin. × 210.

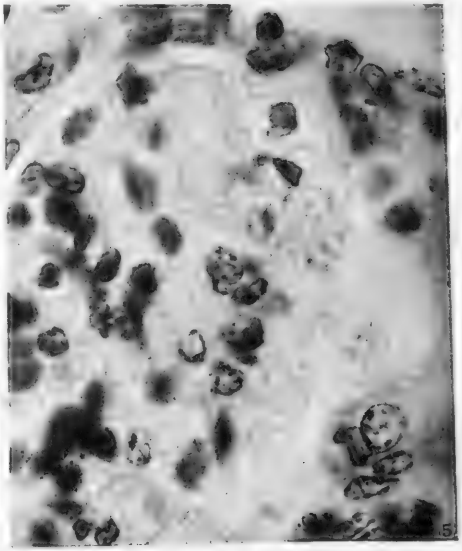
FIG. 21. The two glomeruli to the right show a fairly extensive accumulation of round cells and fibroblasts with an irregular extension into the labyrinth. The glomeruli to the left are practically normal. Hematoxylin and eosin. × 210.





(Pearce: Klosiella Infection of the Guinea Pig.





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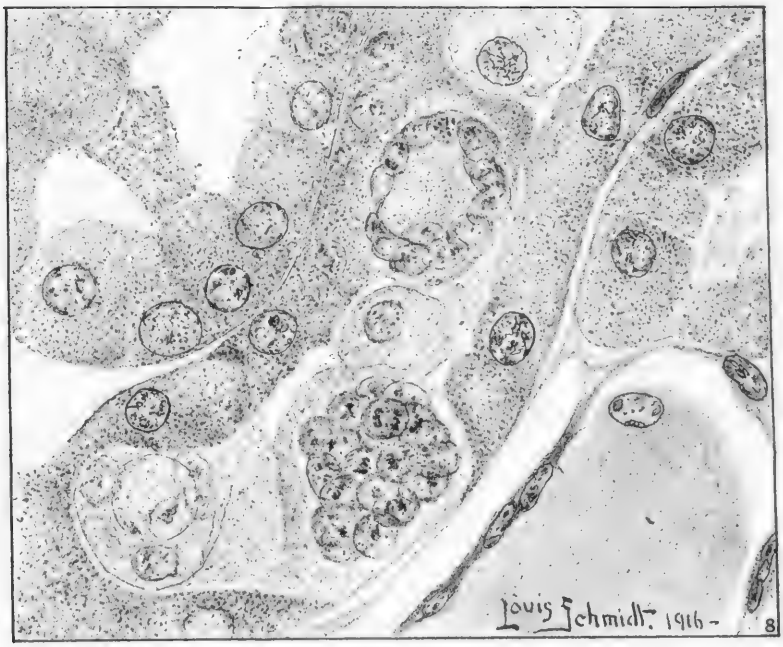

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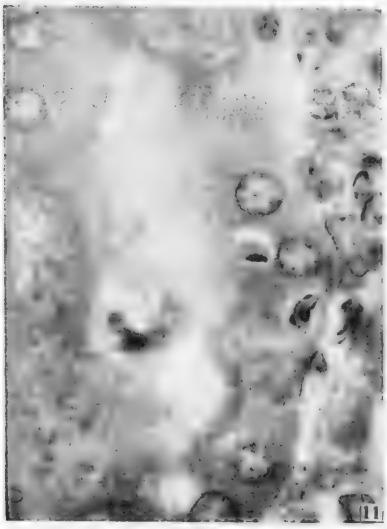

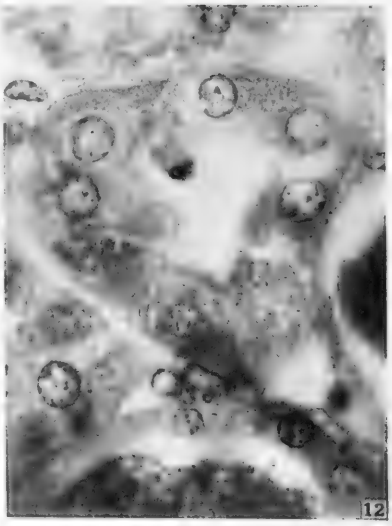

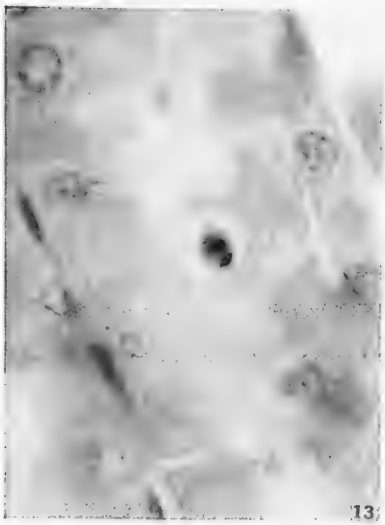

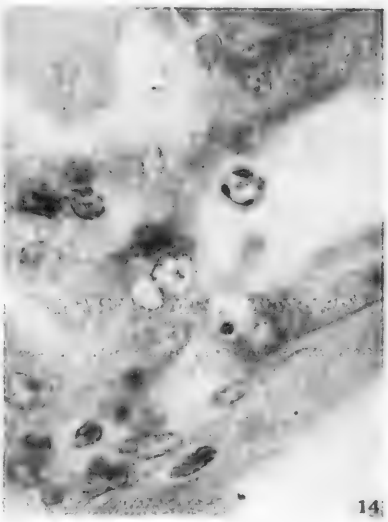

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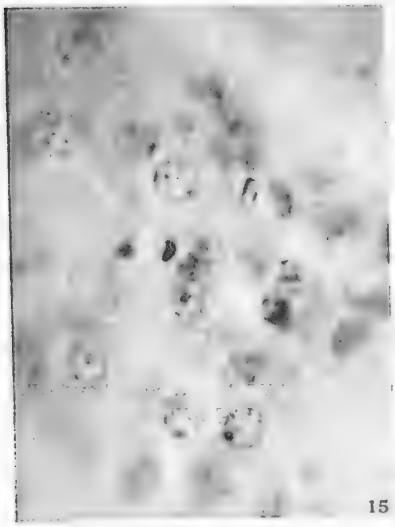

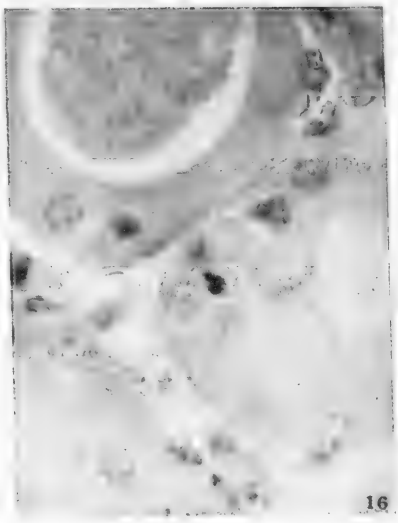

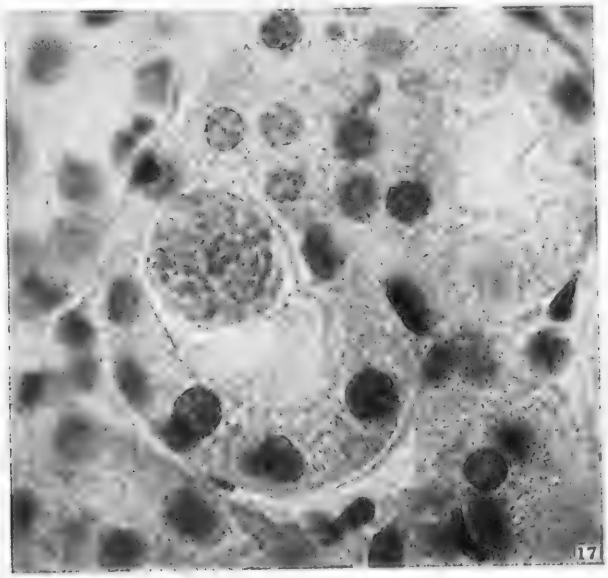

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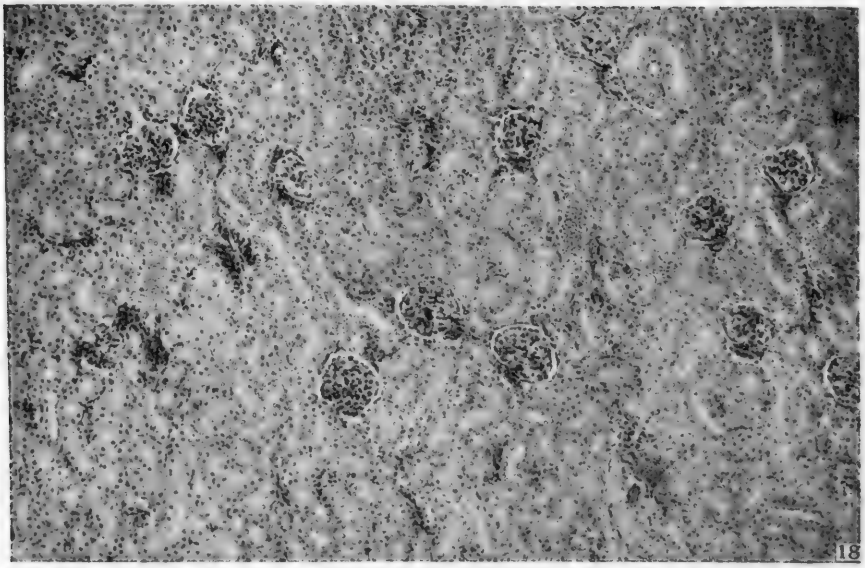
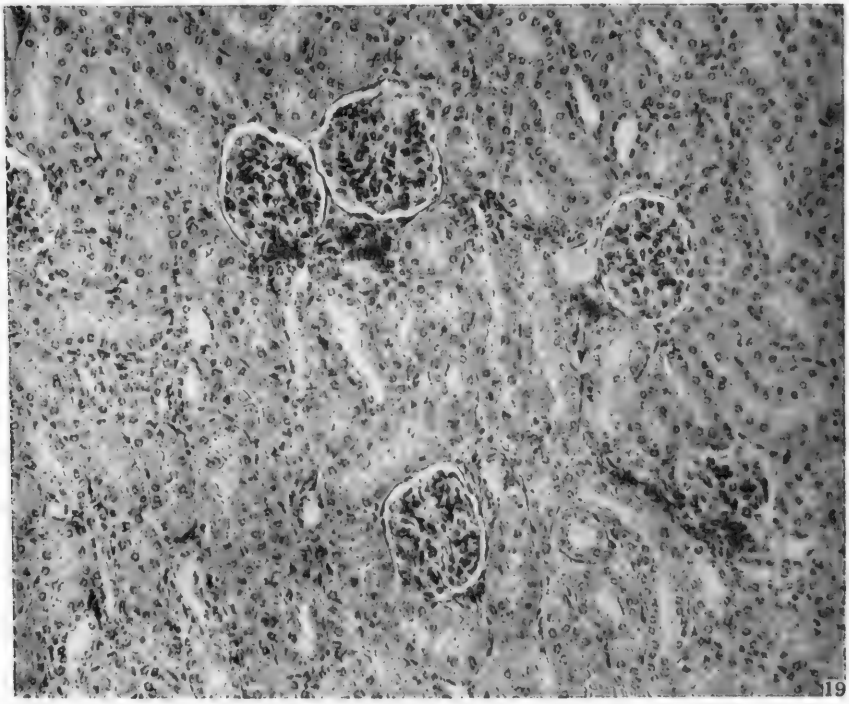
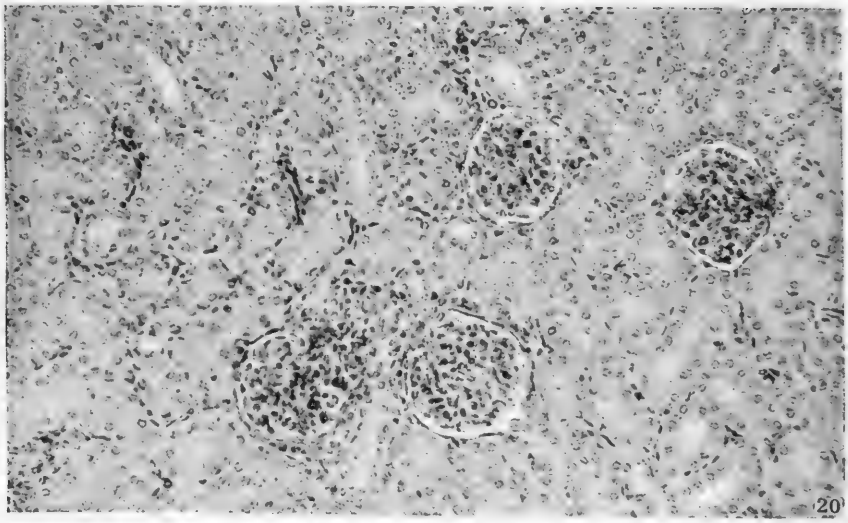




(Pearce: Klosiella Infection of the Guinea Pig.)



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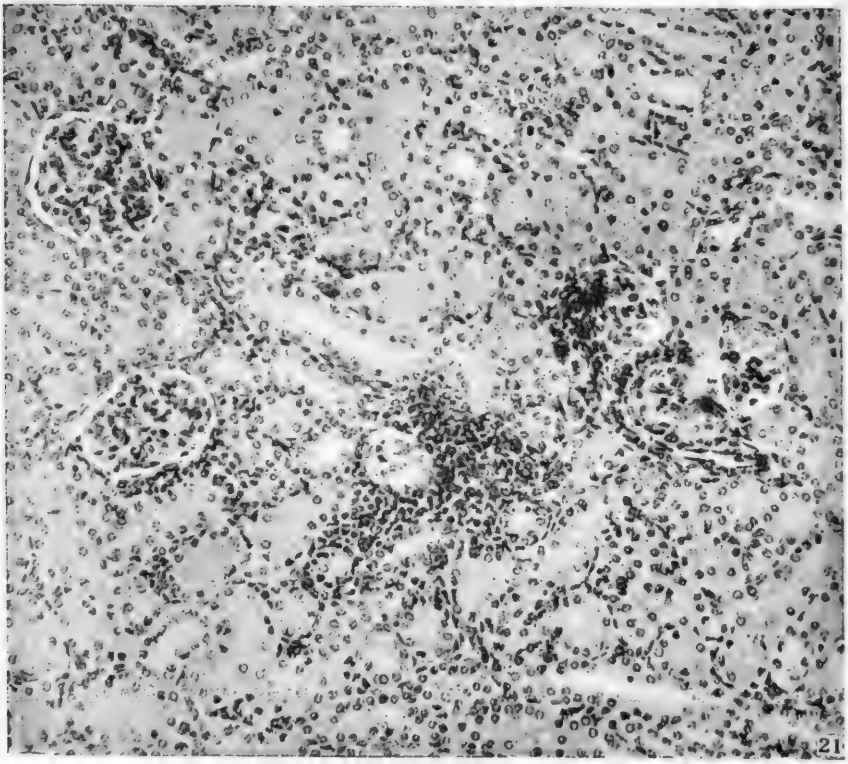

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(Pearce: Klosiella Infection of the Guinea Pig.)

CHEMOPATHOLOGICAL STUDIES WITH COMPOUNDS OF ARSENIC.

IV. The Character and Distribution of Renal Injury Produced by Arsenicals as Indicated by the Processes of Repair.

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Plates 71 to 76.

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In previous papers[1] attention was directed to some of the striking differences in the gross and histological changes produced in the kidneys of dogs by lethal doses of certain compounds of arsenic. Upon the basis of these observations it was pointed out that arsenicals could produce a so called tubular as well as a vascular injury of the kidney and that innumerable combinations of these two fundamental forms of tissue injury were obtainable through the use of arsenical compounds of different chemical constitution.

In order to obtain additional information as to the character and location of the specific renal injury produced by these arsenicals, as well as a knowledge of the subsequent processes of organic repair, we have extended our experiments to a study of tissue changes in the kidneys of animals given sublethal doses of these substances.

EXPERIMENTAL.

In the following experiments guinea pigs were used, as in our experience the kidneys of these animals are as free from spontaneous lesions as those of any animal available for the purpose. However, it should be mentioned in this connection that in the kidneys of some guinea pigs we have found certain pathological changes that seem to be associ-

[1] Pearce, L., and Brown, W. H., *Jour. Exper. Med.*, 1915, xxii, 517, 525.

ated with the presence of a renal parasite.[2] The relative importance of this finding in interpreting the various lesions in the processes of repair will be considered in the discussion.

In our previous experiments[1] dogs were used because the comparatively large size of their kidneys showed gross changes advantageously. However, as we stated then, the histological changes were controlled by experiments carried out with guinea pigs. The gross and microscopic changes in the kidneys of both dogs and guinea pigs following lethal and sublethal doses of these arsenicals were practically identical with but one exception, that of arsenophenylglycine, to which we shall refer at greater length in discussing the results obtained with this compound.

Sterile solutions of arsenious acid, arsenic acid, sodium cacodylate, salvarsan, neosalvarsan, arsacetin, arsenophenylglycine, and atoxyl were injected intraperitoneally into adult male guinea pigs. With each compound, one sublethal dose was given and the animal killed after 2 to 5 days. In addition, with the exception of the salvarsan experiment, other guinea pigs were given two to five small doses of the drug and killed in 3 to 19 days. In a few instances the animal died within 24 hours after receiving a repeated dose. In these guinea pigs an acute lesion complicates the one of longer duration. We were guided in the determination of the size of the sublethal dose by our knowledge of the character and extent of the renal injury caused by lethal amounts of the arsenical in question. We tried always to inject a non-fatal dose, yet one large enough to cause definite renal injury.

Arsenious Acid.

The acute injury to the kidney produced by large doses of arsenious acid is primarily one of congestion and dilatation of the blood vessels with hemorrhage; the tubular epithelium is degenerated with occasional slight cell necrosis and usually there is a voluminous granular precipitate in the glomerular capsule and the tubules. The restoration to normal after a moderate dose of arsenious acid is very rapid and there is but slight evidence of the previous injury (Fig. 1). This consists of large, rather irregular glomeruli with somewhat

[2] Pearce, L., *Jour. Exper. Med.*, 1916, xxiii, 431.

shrunken tufts and a distinct although slight increase in tuft nuclei. About the base of the glomeruli are small accumulations of fibroblasts and round cells (Fig. 1) which occasionally slightly invade the adjacent labyrinthine tissue. Nowhere have we seen any distortion or alteration in the usual architecture in either the cortex or the medulla. The tubular epithelium shows only slight degenerative changes with no evidences of tubular necrosis or regeneration.

Practically the same microscopic picture is found in Guinea Pigs 1, 2, 3, and 4 of this series (Table I), irrespective of the difference in size and number of doses given or the length of survival of the animal. The changes in Guinea Pig 2 are relatively more marked than in the others, although they are essentially of the same type.

TABLE I.

Arsenious Acid.

Animal No.	Day of experiment and dose of drug in mg. per kilo of body weight.					Doses.	Days survived.	Termination.
	1	2	5	6	11			
1	5					1	3	Killed.
2	3	3		10		3	8	"
3	10		10		10	3	12	Died.
4	8		5			2	6	"

The proliferative changes in the kidney, therefore, after sublethal injections of arsenious acid are extremely slight and are confined to slight increase of nuclei in a somewhat shrunken glomerular tuft and to a slight accumulation of fibroblasts and round cells about the base of the glomeruli. There are no mitoses in the tubular epithelium, indicating a previous injury of this tissue with subsequent regeneration.

Arsenic Acid.

The gross and microscopic pictures of the kidneys of guinea pigs poisoned with lethal doses of arsenic acid are practically the same as those of arsenious acid. Vascular injury is the predominating change and there is but slight tubular necrosis. However, the study of the kidneys of guinea pigs that have received sublethal doses of arsenic

acid reveals characteristic changes that differentiate them rather sharply from those of arsenious acid. In the inner half of the cortex and in the boundary zone there is a considerable amount of young fibroblastic (connective) tissue extending for the most part along the medullary rays, but also invading the labyrinth to an appreciable degree. Some of the straight and convoluted tubules in these areas are compressed, but many more are dilated and may contain a granular precipitate (Fig. 2). The epithelium of some of these tubules shows degeneration and is flattened and cuboidal in type. In others it is basophilic and evidently of recent formation. Occasionally mitoses are seen. A few of the tubules in these areas appear as more or less solid cords of recently formed epithelial cells, which stain an intense blue with hematoxylin. The glomeruli in the kidneys of this series are practically normal.

TABLE II.

Arsenic Acid.

Animal No.	Day of experiment and dose of drug in mg. per kilo of body weight.							Doses.	Days survived.	Termination.
	1	2	3	5	6	8	10			
2	2		4		4	10	10	5	13	Killed.
3	8							1	6	"
6	10	10		10				3	7	"

In Guinea Pig 2 (Table II), which received five doses of arsenic acid and survived 13 days, many of the blood vessels in the boundary zone are somewhat thickened. In certain glomeruli some of the capillary walls are apparently thickened also, but this is such an irregular finding and one that may be so variously interpreted that we hesitate to lay much emphasis upon it. The fibroblastic proliferation in the inner cortex and boundary zone is quite characteristic in this animal (Fig. 2). The amount of connective tissue proliferation in Guinea Pig 3 is less than in Guinea Pig 2, but the location and arrangement is the same. In Guinea Pig 6, on the other hand, there is slightly more connective tissue than in Guinea Pig 2, which may be due to the fact that Guinea Pig 6 survived the administration of three comparatively large doses of arsenic acid for 7 days.

In contrasting arsenious and arsenic acid, which resemble each other so closely in the character of the acute renal lesion, it appears that there are distinct differences in their action which are revealed in the process of repair. With arsenious acid, proliferative changes are almost wanting and are practically exclusively confined to the slight increase in nuclei of the glomerular tuft and to slight fibroblastic accumulation about the base of the glomeruli. With arsenic acid, on the other hand, proliferative activity of fibroblasts is appreciably more marked, and is associated with distortion of the tubules especially in the inner half of the cortex and boundary zone and with distinct (although slight) regeneration of tubular epithelium.

Sodium Cacodylate.

TABLE III.

Sodium Cacodylate.

Animal No.	Day of experiment and dose of drug in mg. per kilo of body weight.				Doses.	Days survived.	Termination.
	1	2	5	6			
2	50	100		1,000	3	7	Died.
3	400		1,000		2	6	"
4	700				1	2	Killed.
5	700		700		2	6	Died.
6	500	500			2	3	"

The kidneys of guinea pigs poisoned with lethal doses of sodium cacodylate belong to the red type and resemble those of arsenious and arsenic acid. In the kidneys of guinea pigs which have survived 2 to 7 days, as is shown in Table III, we find practically the same slight proliferative activity that was observed in the animals which received sublethal doses of arsenious acid. There are occasional small accumulations of fibroblasts and round cells about the glomeruli, along the medullary rays, and in the boundary zone (Fig. 4). In some of the glomerular tufts there is a distinct increase in leukocytes of the endothelial type. There is nothing comparable to the fibroblastic proliferation with consequent distortion of tubules that was comparatively conspicuous in the animals poisoned with arsenic acid. However, in addition to the accumulation of fibroblasts there is more

necrosis of the tubular epithelium than with either arsenious or arsenic acid, and mitoses may be found in both the convoluted and the straight tubules.

There is but little difference, histologically, in the kidneys of the various guinea pigs of this series (Table III). In Guinea Pig 2 there are perhaps more and slightly larger accumulations of fibroblasts. Mitoses are found in the tubular epithelium of all five animals, but they are more numerous in Guinea Pig 6.

There is scarcely any fibroblastic proliferation, therefore, in the animals poisoned with sublethal doses of sodium cacodylate as well as with arsenious acid,—in this respect differing from those poisoned with arsenic acid. But, in addition, there is distinct reparative activity on the part of the tubular epithelium, indicating that this tissue has been injured by the drug, and in this respect differing from guinea pigs poisoned with sublethal doses of both arsenious and arsenic acids, in which the tubular epithelium is but little affected.

Salvarsan.

We have but one instance in which a sublethal dose of salvarsan was given and the animal allowed to live for more than 24 hours. This guinea pig received 150 mg. of the drug per kilo of body weight and was killed 48 hours after its administration.

The acute salvarsan kidney is a red kidney. In this one example of an early stage in the process of repair the glomeruli are large and rather irregular. The tufts on the whole are not shrunken and there is an accumulation of endothelial leukocytes in some of them. Many of the walls of the tuft capillaries are hyaline and distinctly thickened. About the base of some of the glomeruli there is a collection of fibroblasts and round cells which in some instances extends slightly outward into the labyrinth between the convoluted tubules. In the boundary zone and along the inner portion of the medullary rays are small irregular patches of young fibroblasts (Fig. 5), but there is no distortion of the tubular structures. The tubular epithelium is markedly degenerated but there is no definite epithelial necrosis, and since we have seen no mitoses we may infer that the tubular epithelium was not seriously injured. It should be stated in this

connection, however, that this animal received the dose of salvarsan intraperitoneally and that the compound was absorbed relatively slowly, for when the animal was killed 2 days after injection, the abdominal cavity contained a quantity of unabsorbed drug.

The evidences of repair, seen in the kidneys of guinea pigs after salvarsan injection, are therefore chiefly the changes in the glomeruli and the irregular proliferation of fibroblasts about the glomeruli and in the boundary zone.

Neosalvarsan.

The acute neosalvarsan kidney resembles those of arsenious acid and salvarsan. In Guinea Pig 1, killed on the 2nd day of survival, as shown in Table IV, we find definite signs of a proliferative activity

TABLE IV.

Neosalvarsan.

Animal No.	Day of experiment and dose of drug in mg. per kilo of body weight.		Doses.	Days survived.	Termination.
	1	5			
1	300		1	2	Killed.
2	190	500	2	6	Died.

in the kidney in addition to the acute lesion which is still present. About the base of the glomeruli are small accumulations of fibroblasts and round cells. In addition there is a slight diffuse infiltration of the same character along the medullary rays in both cortex and medulla which in some areas invades the adjacent labyrinthine tissue. The glomeruli themselves are extremely irregular; some are of normal size, while others are markedly shrunken with a contracted tuft filling approximately three-quarters of the capsular space. The tubular epithelium is considerably degenerated and in the inner portion of the cortex there is some individual cell disintegration of the epithelium of the loops of Henle and a slight degree of necrosis. A few mitotic figures are seen.

The changes in Guinea Pig 2 are of the same general character as those in Guinea Pig 1, although of a lesser degree. This may be

due to the much smaller initial dose. The capsules of Bowman and the capillary walls of some of the glomerular tufts are slightly hyaline and thickened. There are small, irregular patches of fibroblasts and round cells about the glomeruli, along the medullary rays, and in the boundary zone. The tubular epithelium is degenerated with occasional slight necrosis. No mitoses are seen.

The changes in the process of repair after neosalvarsan resemble in general those after salvarsan, differing only in the greater irregularity of the glomeruli and the somewhat greater degree of tubular necrosis which is most marked in the inner portion of the cortex. After both salvarsan and neosalvarsan there is a patchy irregular proliferation of fibroblasts in the boundary zone and cortex, but there is no distortion of the tubules as with arsenic acid.

Arsacetin.

TABLE V.

Arsacetin.

Animal No.	Day of experiment and dose of drug in mg. per kilo of body weight.				Doses.	Days survived.	Termination.
	1	5	13	16			
1	125	250			2	6	Killed.
2	100	200	200	200	4	19	"
3	300				1	2	"

The kidneys of dogs and guinea pigs which have received lethal doses of arsacetin are pale and show a most extensive tubular necrosis with a subordinate vascular injury. In the reaction from a non-fatal dose of this compound there is an exceedingly active and prompt regeneration of the epithelium, especially in the loops of Henle, and to a somewhat less extent in the convoluted tubules and the tubules of the medulla (Figs. 6, 7, and 8). The glomerular capillaries are somewhat dilated and there may be a slight and irregular accumulation of fibroblasts and polyblasts about the base of the glomeruli, along the medullary rays, and in the boundary zone. In Guinea Pig 3 (Table V), killed on the 2nd day, there is already marked activity of the tubular epithelium throughout the cortex and to a less extent in

the medulla. There is practically no vascular alteration and no fibroblastic proliferation in this animal. Guinea Pig 1 was killed on the 6th day after having received two doses of arsacetin. The kidney sections of this animal show a moderate regeneration of the tubular epithelium, almost exclusively confined to the loops of Henle and the medullary tubules. About the base of some of the glomeruli there is a very slight degree of fibroblastic proliferation. Four moderate sized doses of arsacetin were given to Guinea Pig 2 in 16 days, and the animal was killed on the 19th day (Table V). In this animal the regenerative activity of the tubular epithelium has reached a remarkable degree. Mitotic figures are found in practically every microscopic field (4 mm. Zeiss objective; No. 4 ocular) and they are particularly numerous in the straight tubules of the cortex and medulla (Figs. 6, 7, and 8). Many of the tubules along the medullary rays show entirely new epithelium and some of these tubules are almost solid masses of new cells. Degeneration with some necrosis and desquamation of cells is still present in some of the convoluted tubules and limbs of Henle and there are many hyaline casts in the boundary zone and medulla. In the boundary zone, along the medullary rays, and about the base of some of the glomeruli there is a rather diffuse proliferation of fibroblasts and infiltration of polyblasts which irregularly invade the labyrinth in some areas. Several of the renal tubules of this animal contain the parasite which we have referred to and which must be taken into consideration in interpreting the proliferative changes about the glomeruli.

Atoyxl.

The acute lesion in the kidneys of dogs and guinea pigs poisoned with lethal amounts of atoxyl is predominantly tubular, but the vascular changes are by no means inconspicuous. In guinea pigs which have received sublethal doses of atoxyl, there is a rapid attempt at regeneration of the tubular epithelium which is shown in both animals of this series (Table VI). Many of the straight tubules are dilated and lined with low cuboidal basophilic staining cells (Fig. 9), and there are numerous mitoses in both cortical and medullary epithelium (Figs. 9, 10, and 11). There is a well marked leukocytic exudate

in the interstitial tissue as well as in some of the tubules of Guinea Pig 3 (Fig. 11); it is less prominent in Guinea Pig 1. In addition to the marked epithelial necrosis with regeneration and the cellular exudate caused by the injection of atoxyl, there is a definite fibroblastic proliferation along the medullary rays, in the boundary zone, and in the upper medulla (Figs. 11 and 12).

The territorial distribution of fibrosblastic proliferation in Guinea Pig 3 corresponds with that in Guinea Pig 1. The process, however, is distinctly more pronounced, as might be expected, the animal having received three doses of atoxyl in 8 days. The fibroblasts are somewhat diffusely distributed throughout the lower edge of the cortex, and along the boundary zone and upper medulla. Some of the tubules in the area of fibroblastic proliferation are compressed,

TABLE VI.

Atoxyl.

Animal No.	Day of experiment and dose of drug in mg. per kilo of body weight.			Doses.	Days survived.	Termination.
	1	2	5			
1	100			1	3	Killed.
3	50	50	50	3	8	"

while others are slightly dilated (Figs. 9 and 12). The glomeruli are irregular in both animals. Some are quite large, others are shrunken. Many of the tuft capillaries are dilated and the walls moderately thickened. About the base of the glomeruli are slight fibroblastic accumulations which in a few areas invade the adjacent labyrinth.

The process of repair in the kidneys of guinea pigs poisoned with sublethal doses of atoxyl resembles that of arsacetin in the prompt and marked regeneration of tubular epithelium. Moreover, there is a definite proliferation of fibroblasts which recalls the changes caused by arsenic acid, and in addition there is a well marked exudation of polymorphonuclear leukocytes into the interstitial tissue and tubules.

Arsenophenylglycine.

Arsenophenylglycine acts somewhat differently in guinea pigs and in dogs. With lethal amounts of the drug the kidneys of dogs and guinea pigs are both pale, with a predominant tubular necrosis and a relatively subordinate vascular injury. In the guinea pig, however, after a comparable sublethal dose of the compound there is but little tubular necrosis and but slight vascular injury. Consequently, after such a dose of arsenophenylglycine the process of repair in the kidney consists only in recovery from a parenchymatous and fatty degeneration of the tubular epithelium with now and then a mitotic figure.

TABLE VII.

Arsenophenylglycine.

Animal No.	Day of experiment and dose of drug in mg. per kilo of body weight.		Doses.	Days survived.	Termination.
	1	5			
1	250		1	2	Killed.
2	250		1	5	"
3	100	50	2	6	Died.
4	400		1	3	Killed.
5	300	300	2	6	Died.
6	500		1	4	Killed.

Such a course of events is followed after a single or a repeated sublethal dose (Table VII). In Guinea Pig 1, for instance, killed on the 2nd day, degeneration of tubular epithelium is marked, but there is practically no cell necrosis, and no mitotic figures are seen. Guinea Pig 2 received the same amount of arsenophenylglycine as Guinea Pig 1, but was not killed until the 5th day. The epithelial changes in the two animals are almost identical. In Guinea Pig 2 there is an increase of tuft nuclei, mostly of the polymorphonuclear variety, and about the base of some of the glomeruli are slight accumulations of fibroblasts. Guinea Pig 3 received two small doses of the compound, and the alterations here are much the same as in the first two animals. The leukocytic cells infiltrating the glomerular

tuft are mostly eosinophilic; there is a very irregular thickening of the wall of the glomerular capillaries and an extremely irregular and slight distribution of round cells and fibroblasts about the base of some of the glomeruli, along the straight vessels, and in the labyrinth. The tubular epithelium is swollen and granular and there is some necrosis of a disintegrative character in the epithelium of the outer cortex. An occasional mitotic figure is seen.

The acute changes in the kidneys of guinea pigs after sublethal doses of arsenophenylglycine consist almost entirely of parenchymatous and fatty degeneration of the tubular epithelium with but little cell necrosis, differing in this respect from a similar sublethal injury in the kidneys of dogs. The processes of repair after such an injury are, therefore, comparatively simple and do not involve any appreciable degree of cell regeneration. There is only a slight and very irregular interstitial fibroblastic proliferation.

DISCUSSION.

A study of the processes of repair in the kidneys of guinea pigs poisoned with sublethal doses of certain arsenical compounds furnishes additional information as to the character and location of the acute injury. The idea, which we have previously suggested, namely, that all arsenicals do not produce a purely vascular type of renal injury, is further substantiated by this series of experiments in which the regeneration of tubular epithelium plays a relatively conspicuous part. The participation of the epithelial tissue, however, is by no means the dominant feature in the recovery after sublethal doses of all arsenical compounds, but only after certain particular ones. In others, the acute injury is mainly vascular, and the reaction of the epithelial structures is but slight. Further, the interstitial proliferation of fibroblasts which occurs in a marked degree after injections of various arsenicals may be especially pronounced in the repair following a drug which, in lethal doses, causes but little vascular disturbance, as, for instance, arsacetin. Here, the initial and dominant injury is epithelial; there is only slight demonstrable alteration of the vascular structures. In the process of recovery, however, after the administration of sublethal doses of arsacetin, there may be quite

a marked proliferation of young fibroblasts diffusely distributed (Table V, Guinea Pig 2, and Fig. 8). This is also seen in the repair of the kidney following injections of atoxyl, although here the picture is more complicated, for atoxyl affects the vascular as well as the epithelial tissue of the kidney. The distribution of the proliferating fibroblasts after a more purely vascular injury such as that produced by arsenious or arsenic acid is confined more sharply to the boundary zone, with radiations along the medullary rays to the glomeruli. On the other hand, after arsacetin, the fibroblastic proliferation is more diffusely distributed throughout the cortex, although it may be more numerous in the boundary zone and along the medullary rays.

Taking all these observations into consideration, therefore, it would seem that a toxic agent like arsenic, may, in one or another of its various combinations, injure the vascular, epithelial, or interstitial (connective) tissue of the kidney, if we may judge of such an initial injury by the subsequent processes of repair. The relative distribution and extent of the initial injury may be difficult to determine until one studies various stages of the recovery of the kidney, in which the injured tissues are regenerating. This is particularly true in the case of injury of the connective tissue of the kidney.

After a sublethal dose of arsenious acid which produces an almost pure type of vascular injury in the kidneys, the return to normal is very rapid and there is only a slight fibroblastic proliferation about the base of the glomeruli to indicate a previous injury. Injections of sublethal amounts of salvarsan also cause a proliferation of fibroblasts of a more interstitial and rather patchy character, somewhat greater in extent and amount than with arsenious acid. On the other hand, with arsenic acid, there is a relatively large amount of fibroblastic proliferation, especially in the boundary zone, resulting in the compression or dilatation of some of the tubules, and in addition a slight although definite regeneration of tubular epithelium. Sodium cacodylate in sublethal amounts causes essentially the same slight fibroblastic changes seen after arsenious acid, but a well marked regeneration of tubular epithelium as well. With all these four compounds, arsenious and arsenic acid, salvarsan, and sodium cacodylate, there are slight but fairly regular glomerular changes, consisting of

a slightly swollen tuft and an increase in the tuft nuclei. With neosalvarsan, however, the glomeruli are extremely irregular, some being very large, others contracted and shrunken. There is a slight interstitial fibroblastic proliferation, comparable to that of salvarsan and a slight regeneration of tubular epithelium. After sublethal injections of atoxyl, there is very marked regenerative activity of the tubular epithelium, a leukocytic exudate, and a definite and rather diffuse interstitial proliferation of fibroblasts with dilatation or compression of adjacent tubules. Following a sublethal injection of arsacetin, there is a conspicuous and prompt regeneration of tubular epithelium and a relatively diffuse interstitial proliferation of fibroblasts. Unfortunately, we must omit the experiments of arsenophenylglycine on the guinea pig in this consideration, for the reasons stated above.

It would seem, therefore, that arsenicals which produce an injury that is primarily vascular may lead to only a slight subsequent proliferation of tissue (arsenious acid). However, other arsenicals which produce an acute injury that is vascular, may lead to distinct interstitial proliferation (arsenic acid, salvarsan, and neosalvarsan). Moreover, an arsenical that produces an acute injury that is primarily vascular may also produce injury of the tubular epithelium which in the stages of repair dominates the picture to the exclusion of any extensive interstitial proliferation (sodium cacodylate).

On the other hand, arsenicals that produce primarily an injury of tubular epithelium cause a marked subsequent regeneration of this tissue, and may cause in addition an interstitial fibroblastic proliferation (arsacetin). But an arsenical compound that produces a marked vascular injury in addition to the picture of tubular necrosis may lead to a relatively marked interstitial proliferation as well as the extensive epithelial regeneration (atoxyl).

In connection with the fibroblastic proliferation described in the processes of repair in these kidneys, we must refer to the presence of a parasite in the kidneys of some guinea pigs.[2] This parasite is only occasionally found in guinea pigs, but we believe that it may cause irregular accumulations of fibroblasts and round cells about the base of some of the glomeruli and in the neighboring labyrinthine tissue. These accumulations resemble those seen after sublethal injection

of arsenious acid, but they are much more irregular. Therefore, we wish to be particularly cautious in a final interpretation of our results and to take into consideration the possibility that some of the fibroblastic proliferation we have seen in the kidneys of guinea pigs after sublethal injections of these arsenicals may be due in part to this renal parasite.

We have shown that after acute lethal injury with various arsenicals, two types of kidneys could be distinguished, both grossly and histologically, namely, the red and the pale. Further, in the reaction after a sublethal injury inflicted with these compounds these two main types of kidney alteration may still be differentiated by the processes of repair. The administration of those compounds which produce a predominantly pale kidney is followed by a most prompt and pronounced regeneration of tubular epithelium with a varying degree of diffuse fibroblastic proliferation. The compounds that produce a predominantly red kidney are followed by proliferation of fibroblasts with but slight regeneration of the tubular epithelium. The distribution of the fibroblasts in the reparative stages apparently corresponds in some degree to the initial injury. After a more or less predominant vascular injury the fibroblasts are usually found about the glomeruli, along the medullary rays, and in the boundary zone. With other compounds that show little evidence of an acute vascular injury, the connective tissue injury may be more pronounced, and in recovery from such an injury the fibroblasts are diffusely distributed throughout the cortex and medulla in a typical interstitial manner. But, just as there are innumerable combinations of vascular and tubular and probably connective tissue injury in the acute arsenic kidneys, so there are innumerable combinations of the different tissue elements in the processes of repair. Hence, we suggest that the character and distribution of renal injury produced by arsenical compounds as indicated by the processes of repair are bound up in the chemical constitution of these compounds. Further, that each particular compound as far as the kidney is concerned, acts as a more or less specific toxic agent, as shown by the character and distribution of the renal lesions.

SUMMARY.

1. The processes of repair in the kidneys of guinea pigs after sublethal doses of certain arsenical compounds indicate that all arsenicals do not produce a purely vascular type of renal injury.

2. While some arsenicals produce a predominantly vascular injury and others produce a predominantly tubular injury, both these tissue elements are undoubtedly always affected, although in varying proportion. In addition, the interstitial connective tissue is probably always affected. The diffuse proliferation of this tissue may be relatively conspicuous in the processes of repair after arsenicals that cause but slight vascular injury.

3. All red kidneys do not necessarily show identical pictures during the processes of repair; the same is true of pale kidneys.

4. The mode of action of an arsenical compound as a renal toxic agent is bound up with the chemical constitution of the compound.

EXPLANATION OF PLATES.

The illustrations are all from untouched microphotographs.

PLATE 71.

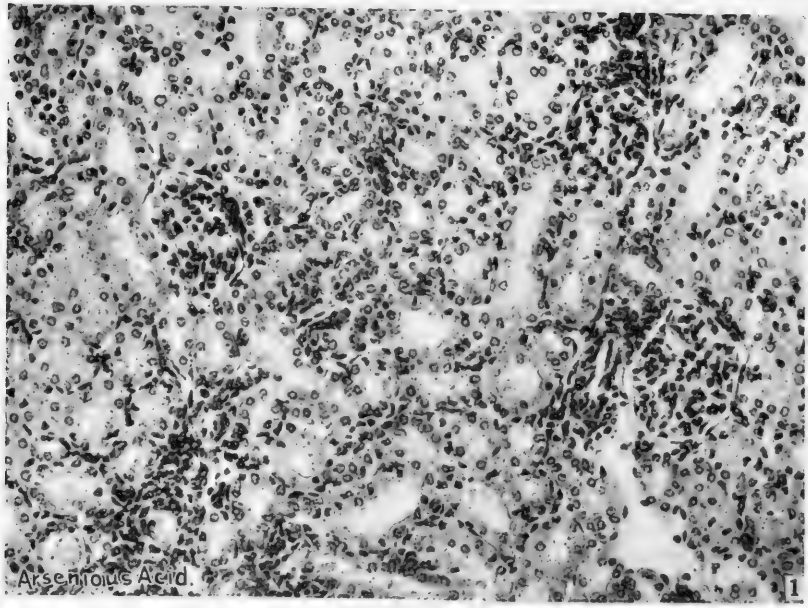
FIG. 1. Arsenious acid. Guinea Pig 2. Section from the outer cortex. There is a regular and definite although slight accumulation of round cells and fibroblasts about the glomeruli. The nuclei of the glomerular tuft show a slight increase. The tubular epithelium is normal. × 210.

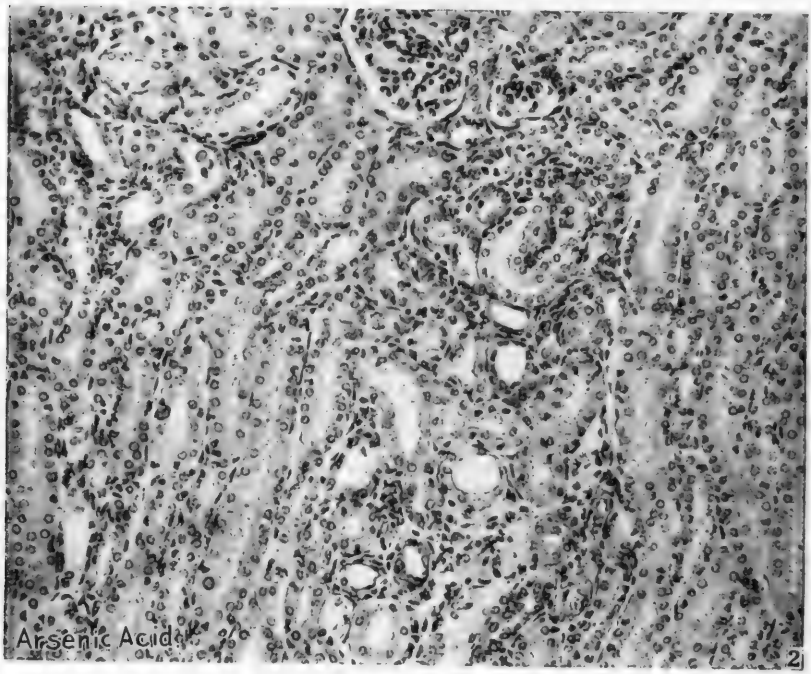
FIG. 2. Arsenic acid. Guinea Pig 2. Section from the inner cortex. There is a slight interstitial proliferation of connective tissue, and the renal tubules in this area are somewhat distorted, some being dilated and others compressed. The tubular epithelium and glomeruli are practically normal. × 210.

PLATE 72.

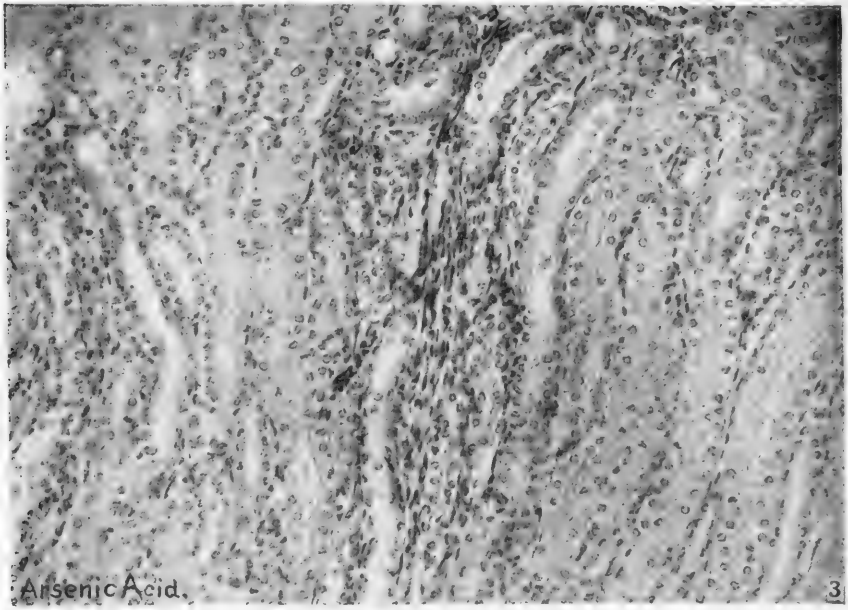
FIG. 3. Arsenic acid. Guinea Pig 6. Section from the boundary zone. There is a small but conspicuous proliferation of connective tissue with consequent dilatation and compression of the renal tubules in this area. The renal epithelium is swollen and shows parenchymatous degeneration. × 210.

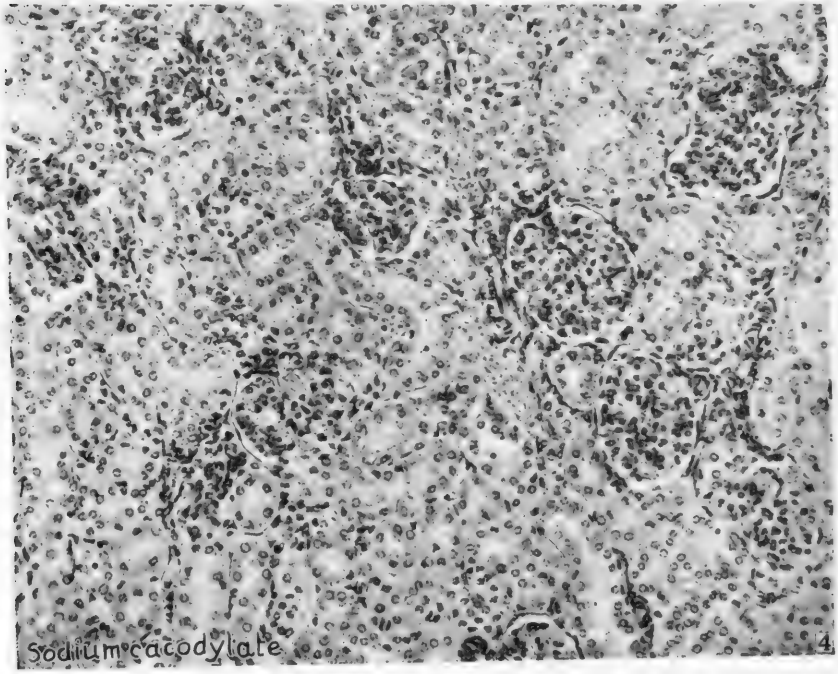
FIG. 4. Sodium cacodylate. Guinea Pig 2. Section from the outer cortex. The glomerular tuft is slightly swollen and shows a slight increase of tuft nuclei. There is a definite and regular although slight infiltration of round cells and fibroblasts around the base of the glomeruli. The renal epithelium is slightly swollen and degenerated. × 210.



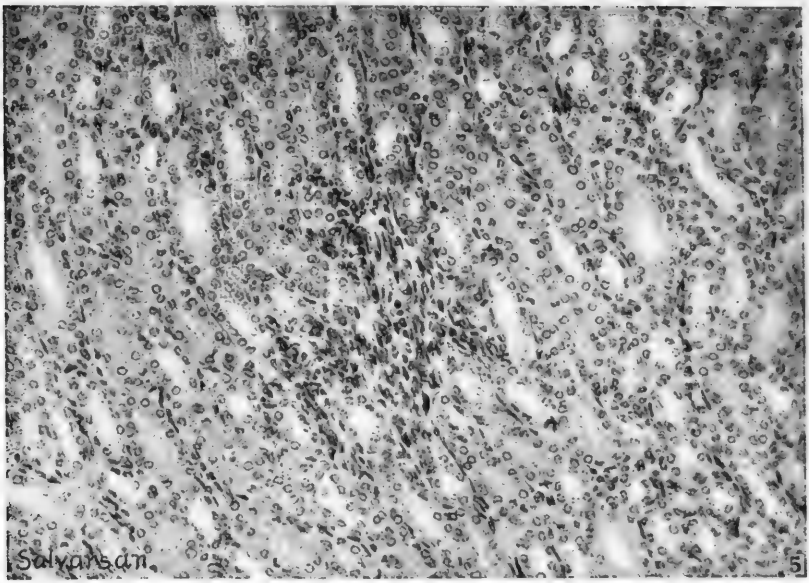


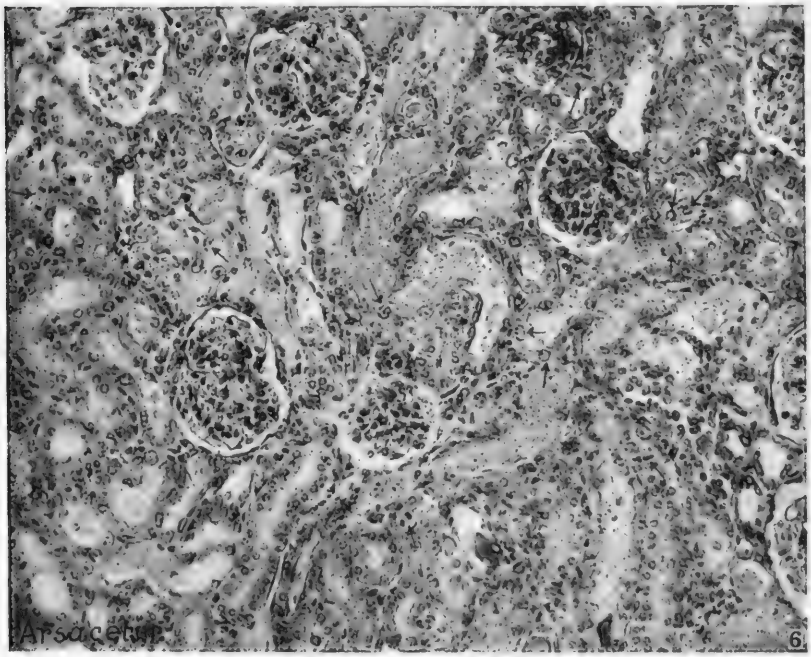
(Pearce and Brown: Renal Injury and Repair.)



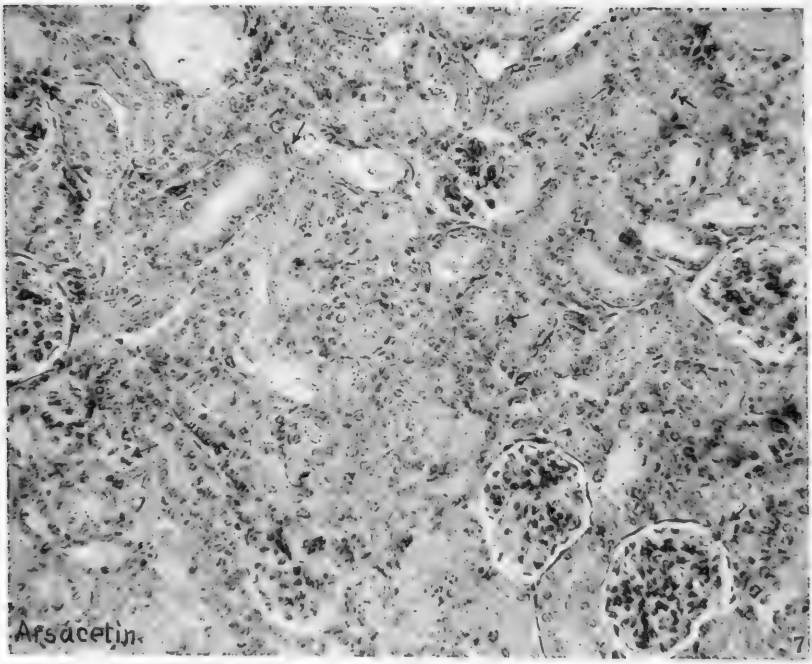


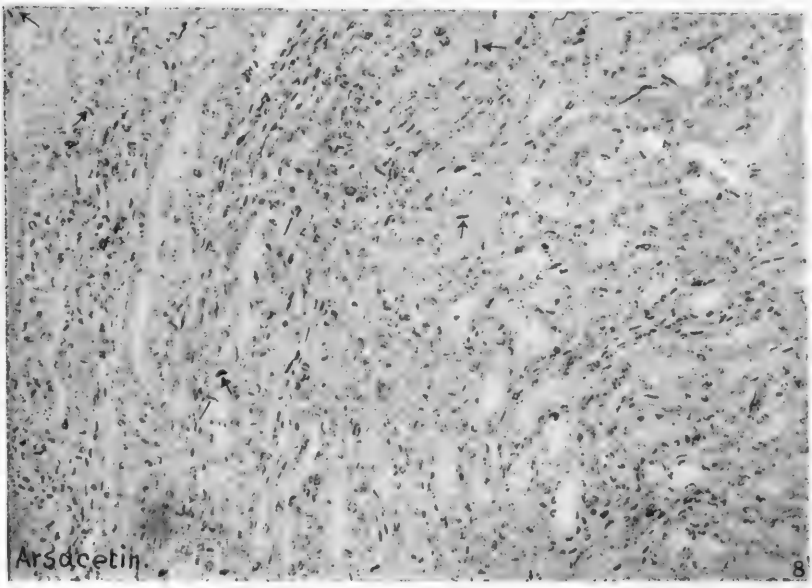
(Pearce and Brown: Renal Injury and Repair.)





(Pearce and Brown. Renal Injury and Repair.)





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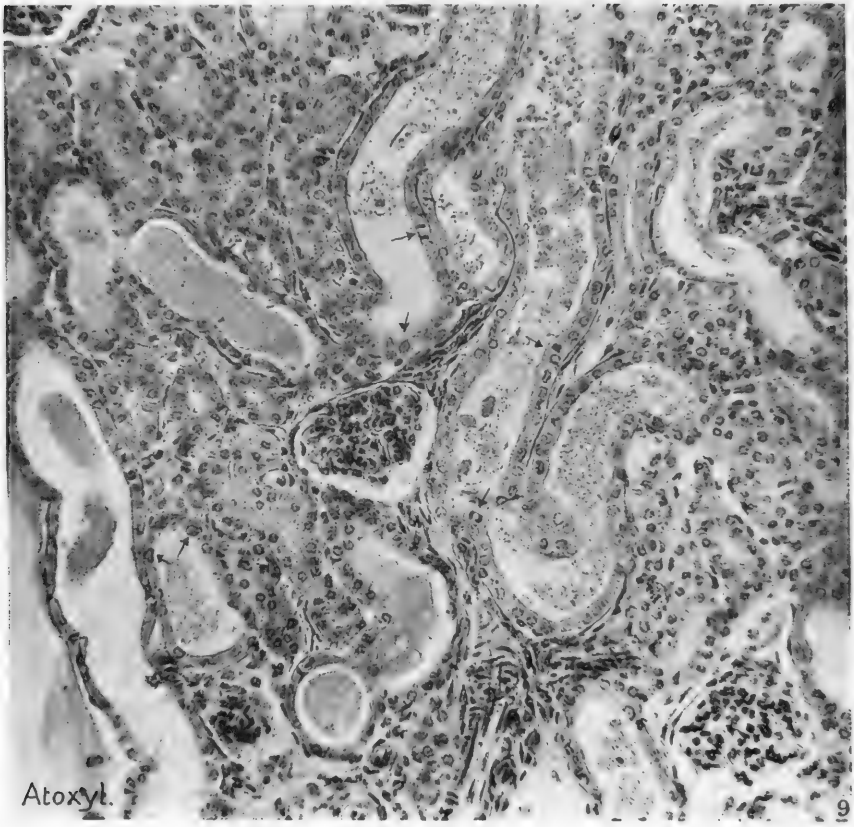

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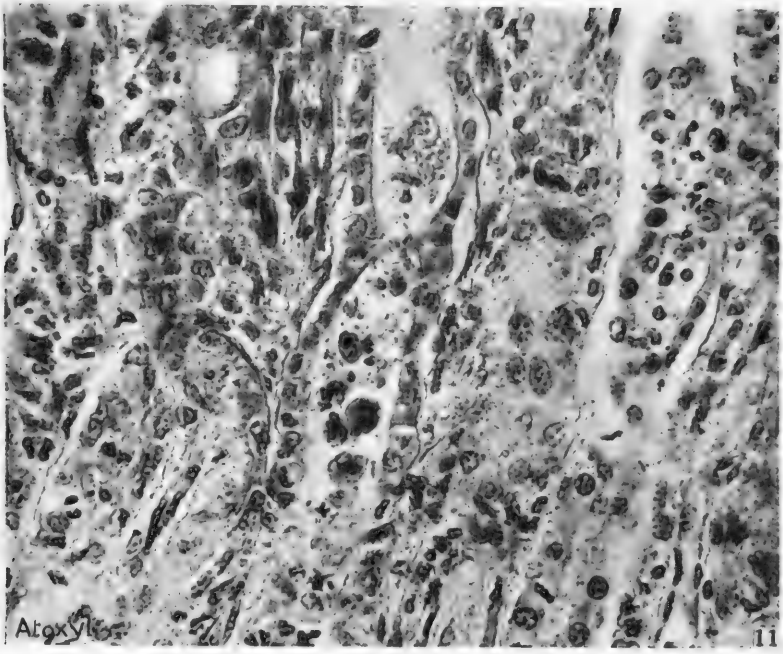

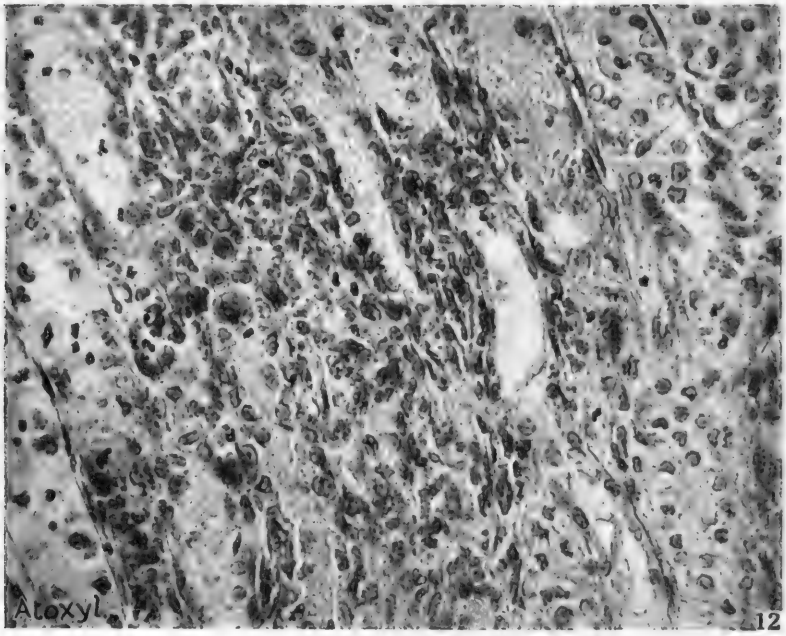

(Pearce and Brown: Renal Injury and Repair.)

PLATE 73.

FIG. 5. Salvarsan. Guinea Pig 1. Section from the boundary zone. There is an irregular patchy proliferation of connective tissue. The renal epithelium is practically normal. × 210.

FIG. 6. Arsacetin. Guinea Pig 2. Section from the outer cortex. There is a marked degeneration and regeneration of the tubular epithelium with numerous mitotic figures. There is a granular precipitate in the lumen of the tubules which appear slightly dilated. The glomeruli are normal in appearance. There is a slight diffuse proliferation of fibroblasts. × 210.

PLATE 74.

FIG. 7. Arsacetin. Guinea Pig 3. Section from the midcortex. There is a moderate degeneration and marked regeneration of the tubular epithelium with numerous mitotic figures. There is a granular precipitate in the lumen of the tubules. × 210.

FIG. 8. Arsacetin. Guinea Pig 2. Section from the upper medulla. There is marked regeneration of the tubular epithelium with numerous mitotic figures. There is a slight but definite interstitial proliferation of fibroblasts. × 210.

PLATE 75.

FIG. 9. Atoxyl. Guinea Pig 3. Section from the inner cortex. There is a conspicuous dilatation of some of the tubules with a granular precipitate in the lumen. The tubular epithelium shows regeneration with numerous mitotic figures. The glomeruli appear normal. × 210.

FIG. 10. Atoxyl. Guinea Pig 1. Section from the outer cortex. There are two adjacent mitotic figures in the epithelium of a convoluted tubule. × 1,470.

PLATE 76.

FIG. 11. Atoxyl. Guinea Pig 3. Section from the boundary zone. There is a conspicuous leukocytic infiltration in the tubules and interstitial tissue. The tubular epithelium shows numerous mitotic figures. There is some interstitial proliferation of the connective tissue. × 378

FIG. 12. Atoxyl. Guinea Pig 3. Section from the boundary zone. There is a diffuse interstitial proliferation of connective tissue. The tubular epithelium shows several mitotic figures. × 378.

EXPERIMENTS WITH POLIOMYELITIS IN THE RABBIT.

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Plates 77 to 80.

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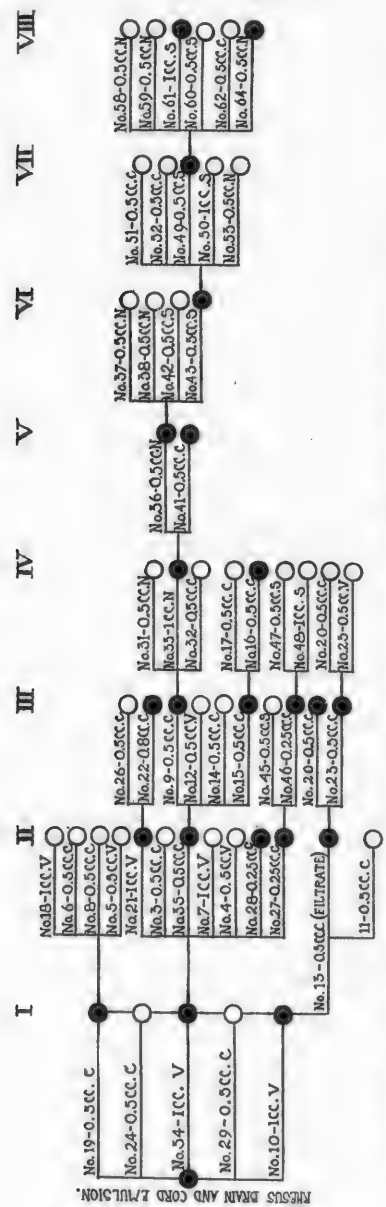
Inoculation of the common laboratory animals with the virus of poliomyelitis has met with so little success that the disease has been generally regarded as exclusively limited to man and the monkey. Attempts have been made to transmit the virus to many animals, but of all the animals tested, positive results have thus far been recorded only for monkeys, rabbits, and, perhaps, guinea pigs.

Krause and Meinicke[1] reported the passage of a strain of virus obtained from a human case through seven generations in rabbits. Lentz and Huntemüller[2] report having successfully transferred the virus from rabbit to rabbit by various methods of inoculation. They found the alterations in the brain and spinal cord to be slight as compared with those in monkeys. On the other hand, Römer and Joseph,[3] Landsteiner and Levaditi,[4] Leiner and von Wiesner,[5] and Flexner and Lewis[6] all failed to transfer the disease to rabbits. The most striking results, perhaps, have been reported by Marks,[7] who carried a strain of poliomyelitic virus derived from a *rhesus* monkey through seven generations of young rabbits varying in weight from 350 to 550 gm. The animals that succumbed developed no paralysis, but died in convulsions. No lesions definitely characteristic of poliomyelitis could be found on microscopic examination. Marks says: "The disease thus produced in rabbits cannot be recognized as poliomyelitis," although he concludes that filtrates of the nervous tissues of monkeys dying from experi-

[1] Krause, P., and Meinicke, E., *Deutsch. med. Wchnschr.*, 1909, xxxv, 1825.
[2] Lentz and Huntemüller, *Ztschr. f. Hyg. u. Infectionskrankh.*, 1910, lxvi, 481.
[3] Römer, P. H., and Joseph, K., *München. med. Wchnschr.*, 1910, lvii, 2685.
[4] Landsteiner, K., and Levaditi, C., *Compt. rend. Soc. de biol.*, 1909, lxvii, 787.
[5] Leiner, C., and von Wiesner, R., *Wien. klin. Wchnschr.*, 1909, xxii, 1698.
[6] Flexner, S., and Lewis, P. A., *Jour. Exper. Med.*, 1910, xii, 227.
[7] Marks, H. K., *Jour. Exper. Med.*, 1911, xiv, 116.



TEXT-FIG. 1. Chart showing the progress of the virus through eight generations of rabbits.
C, intracerebral inoculation; V, intravenous inoculation; N, nasal insufflation; S, injection into sciatic nerve sheath.

mental poliomyelitis are not wholly innocuous to young rabbits. Marks further states that "not all strains of the virus can be transmitted successfully to even a small fraction of individuals of all varieties of domesticated rabbits." This fact, which we have corroborated, may explain the negative results of other investigators.

Römer and Joseph[3] have observed that guinea pigs in the laboratory occasionally die of a paralytic disease. They were unable to transfer poliomyelitic virus from monkeys to guinea pigs, although Römer[8] found that the spontaneous paralysis was due to a filterable virus. Neustaedter[9] claims to have carried a strain from a guinea pig presumably infected through contact with a monkey into one other guinea pig and back again to a monkey.

In the course of our experiments we have inoculated a few guinea pigs. The lesions in those that succumbed were somewhat similar to the lesions seen in the rabbits. This part of our work is not sufficiently advanced to warrant conclusions. Moreover, we have no criterion by which to establish what is and what is not poliomyelitis, except by reproducing the disease in monkeys. It is, therefore, hazardous to affirm or deny the identity with poliomyelitis of these aberrant conditions in other animal species.

The difficulty of obtaining monkeys on account of the war led us to consider other animals that might be susceptible and therefore suitable for experimental purposes. We selected the rabbit because successful results had previously been obtained with these rodents and because rabbits offered a good chance to compare the action of poliomyelitic with rabic virus. We accordingly inoculated rabbits with poliomyelitic virus from a *rhesus* monkey infected by intracranial inoculation with a strain sent us from The Rockefeller Institute for Medical Research by Dr. Harold L. Amoss. We have obtained positive results in young rabbits and have succeeded in transferring the virus from rabbit to rabbit through eight successive generations.

Intracranial inoculations have been used for the most part, although infection has taken place through intravenous injection, also through injection into the sciatic nerve sheath, and even after introduction of the virus into the anterior nares, upon the uninjured nasal mucosa. A few intraperitoneal injections were tried, but with negative results.

[8] Römer, P. H., *Ergebn. d. inn. Med. u. Kinderh.*, 1912, viii, 1.

[9] Neustaedter, M., *Jour. Am. Med. Assn.*, 1913, lx, 982.

All inoculations are by no means successful. Over one-half fail; we obtained positive results in twenty-two rabbits out of a total of fifty-four inoculated in various ways. Of five rabbits inoculated with the virus from the *rhesus* monkey two have failed to show symptoms, although kept under observation for 5 months.

The age incidence of poliomyelitis in man is indicated by the name "infantile paralysis." In our experimental work we obtained positive results in young rabbits only. We failed to infect three full grown rabbits with *rhesus* virus, although this same virus caught in three out of five young rabbits. Furthermore, rabbit virus from the second and third generations was transferred to rabbits 8 weeks old with negative results. Thereafter we used only young animals under 6 weeks old in our experiments. There seems to be a parallel between rabbits and man as far as susceptibility of the young is concerned. These facts seem to furnish a striking example of natural immunity acquired during the period of adolescence.

The incubation period in our observations of twenty-two rabbits has been variable, the shortest being 2 days, the longest, 41. In this respect our experience is similar to that of Lentz and Huntemüller,[2] who found the incubation period to be of uncertain length, sometimes as long as 2 months, but usually between 7 and 11 days. The average period of incubation in our observations was 12 days. Curiously enough, the shortest period, 2 days, as well as the longest, 41 days, was after intracranial injections.

The virus so far has given no evidence of increasing adaptation to rabbits, or of becoming fixed to any degree, the number of failures being as great, and the period of incubation as variable, in the eighth generation as in the first.

The symptoms also vary. They can, however, be divided roughly into two classes: (1) A type which we designate the progressive type, in which the rabbit first becomes inactive, loses weight, and appears weak. This is followed by partial or complete paralysis of one or more of the extremities (Fig. 1), which usually progresses until death. This corresponds somewhat to the syndrome most commonly seen in experimental poliomyelitis in monkeys. The duration of the disease varies from 1 or 2 days to 1 week. The paralysis is usually flaccid, but occasionally it is spastic. Sometimes the paralysis be-

gins locally. As a rule, it is easy to determine that the paralysis is a true palsy and not simply "weakness." (2) The other group of symptoms, which we designate the fulminating type, is more explosive in character, develops suddenly, and terminates in a very short time; it never extends over more than 2 days and usually lasts only a few hours. The overshadowing symptoms are extreme weakness and marked dyspnea. As an illustration of the rapid course of the symptoms of the fulminating type the case of Rabbit 21 is cited. This rabbit was inoculated intravenously on Aug. 18 with 1 cc. of a rich emulsion of virus from a first generation rabbit (No. 34). On Sept. 12, 25 days later, it still appeared to be normal. In about an hour after this observation it was found by one of us lying flat on its side, breathing in slow, labored gasps. There was great general weakness, the rabbit being unable to raise its head, but no paralysis of the extremities was demonstrable. Within a few minutes the animal was dead, the whole syndrome lasting less than an hour. This is an extreme instance of the fulminating type, and is of special interest since it follows a long period of incubation. We have seen combinations of the two types with symptoms resembling Landry's paralysis.

Most of the animals in which the symptoms lasted over 2 or 3 days lost weight rapidly. In some cases the loss of weight was the first observed symptom.

That the rabbit virus is filterable is shown by the protocol of Rabbit 13 of the second generation. This rabbit was inoculated intracranially with 0.5 cc. of a Berkefeld filtrate of brain and cord emulsion from Rabbit 10. Six days later the hind legs became paralyzed, and the animal died 2 days after onset with symptoms of the fulminating type. The unfiltered virus from Rabbit 13 proved infectious for two other rabbits (Nos. 20 and 23).

We have inoculated a monkey with virus from Rabbit 49 of the seventh generation. The monkey died after an incubation period of 4 weeks, with symptoms somewhat resembling the fulminating type seen in the rabbits. The gross pathological findings were congestion of the pia, especially in the region of the medulla, and hyperemia and hemorrhage of the gray matter of the medulla and cord. The microscopic lesions were similar to those seen in the rabbits and not typi-

cal of experimental poliomyelitis in monkeys. The monkey was a South American species which we have since found to be highly resistant to poliomyelitic virus derived from *rhesus* monkeys. This interesting observation is being made the subject of further study.

Flexner found that *Capucinus* monkeys were resistant, although *Cebus*, another South American species, were susceptible, but less so than Old World monkeys. He therefore concludes that the *Platyrrhines* are less susceptible than the *Catarrhines*.[10]

The intracranial inoculations were done by making a small incision in the skin, near the midline, drawing this to one side, and introducing the needle of the syringe directly through the frontal bone, which is thin and soft in young rabbits. The virus is then injected slowly into the region of the lateral ventricle. On withdrawing the needle, the skin slides back and acts as a valve to cover the small opening in the bone.

The emulsion of virus in all cases was made by grinding in a mortar, portions of the brain and cord with salt solution, and filtering through several layers of sterile gauze.

In Table I are briefly summarized the results of the rabbits which succumbed to intracranial inoculation.

Fourteen rabbits inoculated intracranially failed to show symptoms.

The intravenous inoculations were made into the posterior auricular vein with an emulsion of the central nervous matter prepared as above described.

Table II contains a condensed summary of the rabbits which succumbed to intravenous inoculations.

Six rabbits inoculated intravenously failed to show symptoms.

In order to inject the virus into the sciatic nerve sheath, a short incision was made in the skin above the nerve, the tissues were dissected sufficiently to render the nerve visible, and the needle was then inserted into the sheath in the central direction. The emulsion was prepared as above.

Table III gives a condensed summary of the rabbits which succumbed as a result of injection into the sciatic nerve sheath.

Five rabbits inoculated into the sciatic nerve sheath failed to develop symptoms.

[10] Flexner, S., *Jour. Am. Med. Assn.*, 1910, lv, 1105.

TABLE I.

Intracranial Inoculation.

No. of rabbit.	Amount of emulsion.	Rabbit generation.	Period of incubation.	Type of symptoms.*
	cc.		*days*	
19	0.5	I	19	P.
35	0.5	II	5	P.
27	0.25	II	31	F.
28	0.25	II	34	F.
13	0.5	II	6	F.
20	0.5	III	41	F.
23	0.5	III	9	P.
22	0.8	III	10	P.
46	0.25	III	10	Convulsions.
15	0.5	III	2	F.
9	0.5	III	14	P.
16	0.5	IV	13	P.
41	0.5	V	12	P.

* In this and the following tables, the letter P indicates symptoms of the progressive type; F, symptoms of the fulminating type.

TABLE II.

Intravenous Inoculation.

No. of rabbit.	Amount of emulsion.	Rabbit generation.	Period of incubation.	Type of symptoms.
	cc.		*days*	
34	1.0	I	18	F.
10	1.0	I	3	F.
21	1.0	II	25	F.

TABLE III.

Inoculation into the Sciatic Nerve Sheath.

No. of rabbit.	Amount of emulsion.	Rabbit generation.	Period of incubation.	Type of symptoms.
	cc.		*days*	
43	0.5	VI	8	P.
49	0.5	VII	6	F.
61	1.0	VIII	20	F.

In order to introduce the virus into the nose, the following procedure was adopted. The rabbit was lightly etherized, held on its back, and the emulsion of the virus then dropped into the anterior nares from an ordinary medicine dropper, care being taken not to injure the mucosa. There was usually a little sneezing immediately after the fluid was introduced.

Table IV is a condensed summary of the three rabbits which succumbed as a result of intranasal insufflation.

TABLE IV.

Intranasal Insufflation.

No. of rabbit.	Amount of emulsion.	Rabbit generation.	Period of incubation.	Type of symptoms.
	cc.		*days*	
33	0.5	IV	2	F.
36	0.5	V	2	F.
64	0.25	VIII	2	F.

Six rabbits inoculated by intranasal insufflation failed to develop symptoms.

It will be seen that, judging from the limited data at hand, this is a particularly virulent way of infecting rabbits with the virus. The period of incubation is short—only 2 days—and the symptoms in all three cases were of the fulminating type. These represent only three out of nine rabbits tested by introducing the virus into the nose. The other six failed to take. Particular attention was paid to the lungs of the rabbits in this group at autopsy. There was no evidence of pneumonia, or even of congestion of the lungs. On microscopical examination it was found that the medulla was markedly congested, whereas the cord was only slightly affected.

The gross lesions consist of injection of the vessels of the pia, hyperemia of the gray matter of the medulla and cord, and more or less marked edema throughout the brain and cord. The microscopic lesions are distributed rather uniformly throughout the gray matter of the cord and medulla in the progressive type. In the fulminating group (those exhibiting symptoms of respiratory failure without

paralysis of the skeletal muscles), the lesions are more marked in the medulla.

The microscopic lesions consist of capillary congestion, focal hemorrhages into the gray matter, degeneration of the large motor cells, and infiltration with cells of uncertain origin (Figs. 2, 3, 4, 5, 6, 7, and 8). These cells for the most part seem to be proliferated glia cells, and therefore appear to be different from the infiltrating lymphocytes of the lesions of poliomyelitis in man. The congestion of the capillaries and small arterioles is conspicuous. The infiltrating cells when stained with eosin and methylene blue have large, vesicular nuclei with a number of conspicuous chromatin granules. The cytoplasm is scanty and homogeneous. They are scattered throughout the gray matter, and are also grouped in satellite arrangement around the nerve cells, but the perivascular infiltration, so typical of the lesions of poliomyelitis in man and the monkey, is absent in the rabbit. Punctate hemorrhages are numerous, sometimes every vessel in a field being ruptured. The nerve cells show all stages of degeneration. Chromatolysis is common and satellitosis is an almost constant feature. A moderate degree of round cell infiltration is occasionally seen in the meninges. The accompanying plates illustrate the different features of the lesions. The complete picture gives the impression of a severe intoxication of the gray matter of the cord and medulla.

The following are the protocols in brief of the rabbits that succumbed, arranged chronologically, as in Text-fig. 1.

Rabbit 34.—Age 6 wks. Weight 660 gm. 1st generation. 1 cc. of *rhesus* virus intravenously. Incubation period 18 days. Died in the night with no observed symptoms.

Rabbit 19.—Age 5 wks. Weight 600 gm. 1st generation. 0.5 cc. of *rhesus* virus intracranially. Incubation period 19 days. Death in 4 days. Paralysis of front legs with respiratory symptoms.

Rabbit 10.—Age 4 wks. Weight 550 gm. 1st generation. 1 cc. of *rhesus* virus intravenously. Incubation period 3 days. Died in the night with no observed symptoms.

Rabbit 21.—Age 5 wks. Weight 600 gm. 2nd generation. 1 cc. of virus of Rabbit 34 intravenously. Incubation period 25 days. Death in 1 hour. Symptoms very explosive in character; complete prostration and marked dyspnea.

Rabbit 35.—Age 6 wks. Weight 730 gm. 2nd generation. 0.5 cc. of virus of Rabbit 34 intracranially. Incubation period 5 days. Hind legs became

paralyzed. Paralysis remained stationary for 5 days, then showed tendency to improvement. Chloroformed.

Rabbit 27.—Age 3 wks. Weight 290 gm. 2nd generation. 0.25 cc. of virus of Rabbit 34 intracranially. Incubation period 31 days. Death in 1 day. Paralysis and dyspnea.

Rabbit 28.—Age 3 wks. Weight 255 gm. 2nd generation. 0.25 cc. of virus of Rabbit 34 intracranially. Incubation period 34 days. Death in 1 day. Weakness and dyspnea.

Rabbit 13.—Age 4 wks. Weight 540 gm. 2nd generation. 0.5 cc. of Berkefeld filtrate of emulsion of virus of Rabbit 10 intracranially. Incubation period 6 days. Death in 2 days. Paralysis of hind legs and respiratory distress.

Rabbit 20.—Age 5 wks. Weight 645 gm. 3rd generation. 0.5 cc. of virus of Rabbit 13 intracranially. Incubation period 41 days. Death in 1 day. Explosive symptoms; prostration and marked dyspnea.

Rabbit 23.—Age 4 wks. Weight 410 gm. 3rd generation. 0.5 cc. of virus of Rabbit 13 intracranially. Incubation period 9 days. Death in 5 days. General weakness and dyspnea.

Rabbit 22.—Age 5 wks. Weight 620 gm. 3rd generation. 0.8 cc. of virus of Rabbit 21 intracranially. Incubation period 10 days. Death in 7 days. Paralysis progressive.

Rabbit 46.—Age 5 wks. Weight 380 gm. 3rd generation. 0.25 cc. of virus of Rabbit 27 intracranially. Incubation period 10 days. Death in 1 day. Died in convulsions.

Rabbit 15.—Age 6 wks. Weight 520 gm. 3rd generation. 0.5 cc. of virus of Rabbit 35 intracranially. Incubation period 2 days. Symptoms progressed rapidly; respiratory distress and great weakness. Chloroformed.

Rabbit 9.—Age 4 wks. Weight 440 gm. 3rd generation. 0.5 cc. of virus of Rabbit 35 intracranially. Incubation period 14 days. Death in 7 days. Progressive paralysis with respiratory distress.

Rabbit 16.—Age 4 wks. Weight 430 gm. 4th generation. 0.5 cc. of virus of Rabbit 15 intracranially. Incubation period 13 days. Death in 8 days. Symptoms progressive in type with dyspnea.

Rabbit 33.—Age 6 wks. Weight 620 gm. 4th generation. 0.5 cc. of emulsion of virus of Rabbit 9, half the amount dropped in each nostril. Incubation period 2 days. Death in 1 day. Symptoms of the fulminating type with paralysis of hind legs towards the last.

Rabbit 41.—Age 5 wks. Weight 560 gm. 5th generation. 0.5 cc. of emulsion of virus of Rabbit 33 intracranially. Incubation period 12 days. Death in 4 days. Symptoms of progressive type.

Rabbit 36.—Age 4 wks. Weight 380 gms. 5th generation. 0.25 cc. of heavy emulsion of virus of Rabbit 33 in each nostril. Incubation period 2 days. Death in 2 days. Symptoms of fulminating type with paralysis.

Rabbit 43.—Age 5 wks. Weight 450 gm. 6th generation. 0.5 cc. of emulsion of virus of Rabbit 36 injected into sciatic nerve sheath. Incubation period 8

days. Symptoms progressive in type. Complete paralysis on 3rd day. Chloroformed.

Rabbit 49.—Age 4 wks. Weight 310 gm. 7th generation. 0.5 cc. of emulsion of virus of Rabbit 43 into sciatic nerve sheath. Incubation period 6 days. Death in 2 days. Symptoms fulminating in type.

Rabbit 64.—Age 4 wks. Weight 400 gm. 8th generation. 0.25 cc. of emulsion of virus of Rabbit 49 into each nostril. Incubation period 2 days. Death in 2 days. Symptoms of fulminating type.

Rabbit 61.—Age 4 wks. Weight 340 gm. 8th generation. 1.0 cc. of emulsion of virus of Rabbit 49 into sciatic nerve sheath. Incubation period 20 days. Died in night with no observed symptoms.

DISCUSSION AND SUMMARY.

The poliomyelitic virus obtained from an experimental monkey has been passed through eight generations in rabbits. It shows no signs of dying out. On the other hand, it gives no evidence of becoming more pathogenic to the species through successive passage. The period of incubation remains variable and the percentage of takes has not increased. Whether eventually a virus can be obtained which is of heightened virulence to rabbits is problematic.

All inoculations are by no means successful. The animals show great individual differences in susceptibility to the virus, as is evidenced by the fact that out of fifty-four rabbits inoculated, only twenty-two, or about 40 per cent, succumbed. This fact may explain the negative results of other investigators. At several points in the series of experiments it was thought that the strain had died out. As many as six rabbits have been inoculated one after the other before the virus would catch again.

The age of the rabbits is important in considering the susceptibility. From the limited data at our command, adult rabbits are resistant, and there appears to be an abrupt increase in resistance between the age of 6 and 8 weeks; that is, rabbits under 6 weeks are more susceptible to the virus. There seems to be a parallel between the age incidence of this disease in rabbits and spontaneous poliomyelitis in man. The age incidence of poliomyelitis in man is indicated by the term "infantile paralysis."

Several methods of inoculation have proved successful; thus the rabbits have succumbed as a result of introducing the virus directly

into the brain, by injecting it into a peripheral nerve, or directly into the circulation, or by placing it upon the uninjured nasal mucosa.

The symptoms produced show more or less departure from the symptoms of poliomyelitis as seen in the spontaneous disease in man and in the experimental disease in the monkey. There are two distinct pictures recognizable. In one there is paralysis of one or more of the extremities which progresses until death, resembling somewhat the symptoms of the experimental disease in the monkey. This we have designated the progressive type. The other group is included in what we have called the fulminating type. The symptoms are explosive in character, with extreme weakness amounting to prostration, terminating in death in a few hours, attendant upon respiratory failure. The mode of inoculation seems to have little effect upon the type of symptoms produced.

The period of incubation is variable and apparently does not depend upon the method of inoculation. The period varied from 2 to 41 days, with an average of 12 days. The two extremes both occurred after intracranial injection. After intranasal insufflation the incubation period was short, being in each case 2 days, followed by symptoms of the fulminating type. The placing of the virus into the nose seems to be an effective method, but is as uncertain as other routes, as only three out of nine rabbits tested in this manner succumbed. The disease produced by this route was particularly virulent.

The virus shows no tendency to become fixed. The period of incubation is as variable in the eighth generation as in the first, and the virus has shown no tendency towards increasing virulence through successive passage, in these respects differing from the virus of rabies.

We have found the virus to be filterable. An emulsion of the central nervous matter of a rabbit of the first generation passed through a Berkefeld filter, and injected intracerebrally into another rabbit, resulted in death, preceded by symptoms of the fulminating type. Virus (unfiltered) from this rabbit was transferred successfully to two other rabbits.

The lesions, while definite and consistent throughout the series, lack the distinctive features of the pathologic picture of poliomyelitis in man and the monkey. Capillary congestion, punctate hemor-

rhages, degeneration of the motor cells, satellitosis, and more or less cellular infiltration of the gray matter of the cord and medulla are found, but perivascular infiltration is absent and the infiltrating cells are not lymphocytic in character.

One of the most striking features of this investigation is the way in which rabbits and monkeys react to the same virus. The disease in the rabbit presents certain clinical resemblances to the experimental disease in the monkey and also to the spontaneous disease in children. On the other hand, the symptoms show marked variation from those seen in the monkey and in man. The picture has not the same constancy in rabbits and could not in most cases be recognized clinically as poliomyelitis. There are still more marked differences in the pathology. While it is true that the brunt of the attack in the rabbit falls upon the gray matter of the cord and medulla, the appearance of the lesions under the microscope shows such differences from the lesions of experimental poliomyelitis in monkeys, as well as the natural disease in man, as to suggest two distinct infections. It is more reasonable, however, to assume that we are dealing with a modified form of poliomyelitis; that the rabbit reacts differently to the virus than the monkey or man; and that the disease produced in rabbits by us and others is in fact poliomyelitis. So far as we know, no other virus produces such differences in two animal species. Smallpox is so profoundly altered in the cow that it took almost 100 years to prove Jenner's assumption that cowpox is a modified form of smallpox. However, the pock of vaccinia is a correct counterpart both clinically and pathologically of the pock of variola. If the virus of poliomyelitis may be so altered in the rabbit as scarcely to be recognizable, may it not be still more profoundly changed in other animals? The conjecture then arises that poliomyelitis, instead of being limited naturally to man and experimentally to monkeys, may in fact occur in other animals in unnoticed or unrecognized form. If this should prove true, it may be a source of human infection and may help to solve the problem of prevention.

EXPLANATION OF PLATES.[11]

PLATE 77.

FIG. 1. Rabbit 19. Paralysis of extremities.

FIG. 2. Rabbit 19. Medulla. Chromatolysis of the nerve cells. × 515.

PLATE 78.

FIG. 3. Rabbit 9. Lumbar cord, showing capillary congestion and hemorrhage. × 180.

FIG. 4. Rabbit 9. Lumbar cord. Numerous punctate hemorrhages, and marked cell degeneration. × 385.

PLATE 79.

FIG. 5. Rabbit 35. Lumbar cord. Cellular infiltration. × 180.

FIG. 6. Rabbit 9. Medulla. Satellitosis around degenerated nerve cells. × 515.

PLATE 80.

FIG. 7. Rabbit 35. Satellitosis. × 515.

FIG. 8. Rabbit 19. Cervical cord. Degeneration of anterior horn cells with satellitosis. × 385.

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[11] We are indebted to Dr. J. P. Bill for the microphotographic work.

FIG. 1

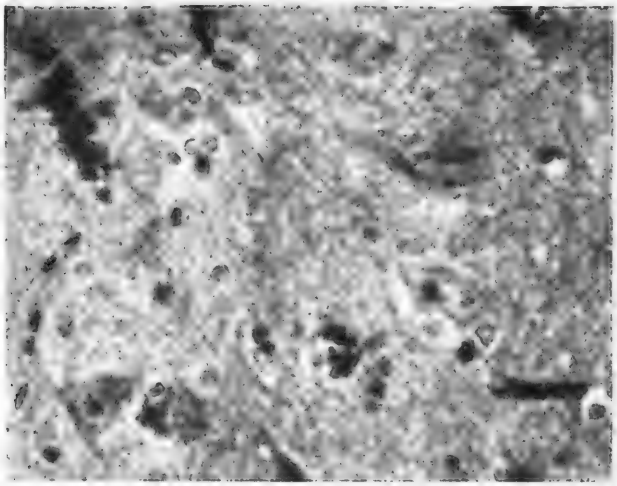

FIG. 2.

(Rosenau and Havens: Poliomyelitis in the Rabbit.)

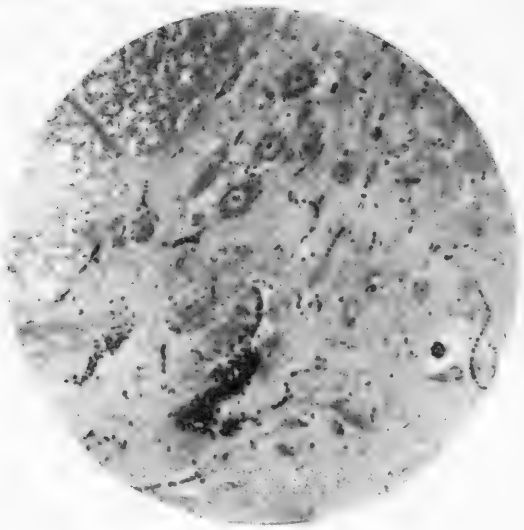

FIG. 3.

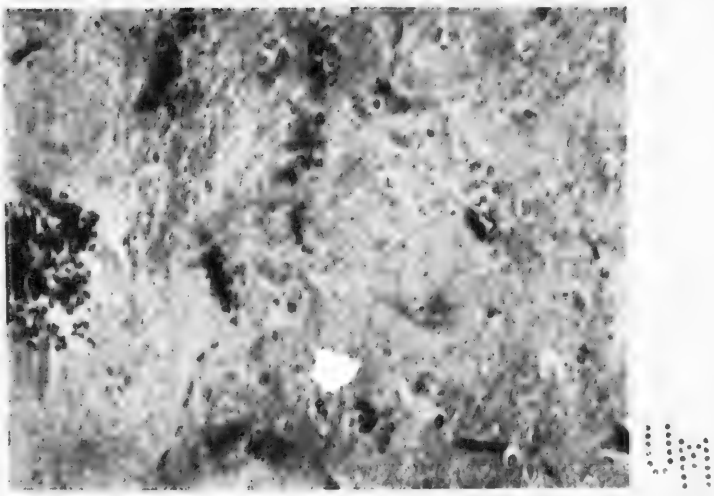

FIG. 4.

(Rosenau and Havens: Poliomyelitis in the Rabbit.)

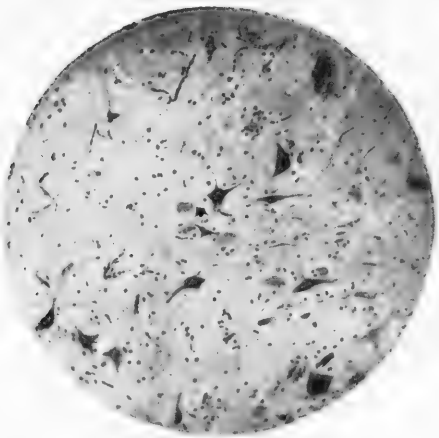

FIG. 5

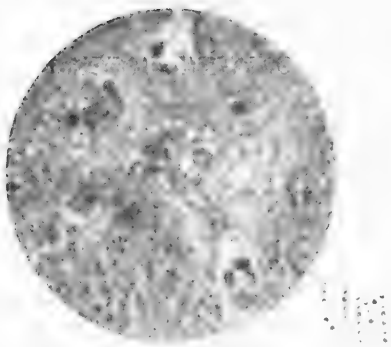

FIG. 6.

(Rosenau and Havens: Poliomyelitis in the Rabbit.)

FIG. 7.

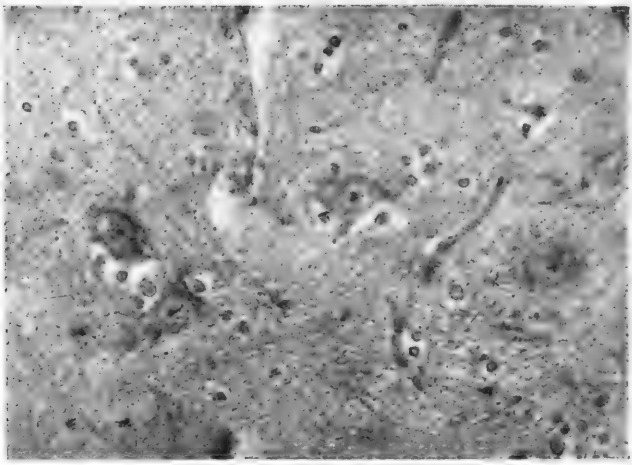

FIG. 8.

(Rosenau and Havens: Poliomyelitis in the Rabbit.)

FEEDING EXPERIMENTS WITH BACTERIUM PULLORUM. THE TOXICITY OF INFECTED EGGS.

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In a previous investigation on the bacteriology of the hen's egg[1] sufficient evidence was acquired to show that the contents of normal fresh eggs are, as a rule, sterile, and that even eggs which have been incubated artificially for 3 weeks remain relatively free from bacterial invasion, provided that they were fresh and clean when placed in the incubator. It was shown, however, that eggs which come from fowls that are permanent bacillus (*Bacterium pullorum*) carriers are often exceptions to the rule.

The carrier problem in bacillary white diarrhea of young chicks has assumed considerable importance in recent years.[2] It has been demonstrated beyond doubt that the mother hen is the permanent source of infection, as the result of acquiring the disease early in her existence, or after full maturity. The permanent seat of infection is the ovary, which in many instances becomes so greatly involved that the ova are discolored and misshapen, and the ovary presents a decidedly pathological appearance. The ova harbor the disease organism. Furthermore, ova which develop into apparently normal yolks frequently carry the organism, *Bacterium pullorum*, to the time of full formation of the egg. Infected eggs produce infected chicks, and although chicks succumb frequently before their embryonic development is completed, a large percentage of them emerge from

[1] Rettger, L. F., *Centralbl. f. Bakteriol., 2te Abt.*, 1913–14, xxxix, 611; *Bull. Storrs Agricultural Experiment Station*, 1913, No. 75.

[2] Rettger, L. F., and Stoneburn, F. H., *Bull. Storrs Agricultural Experiment Station*, 1909, No. 60; 1911, No. 68. Rettger, L. F., Kirkpatrick, W. F., and Stoneburn, F. H., *Bull. Storrs Agricultural Experiment Station*, 1912, No. 74. Rettger, L. F., Kirkpatrick, W. F., and Jones, R. E., *Bull. Storrs Agricultural Experiment Station*, 1914, No. 77.

the shell apparently sound and well. However, they soon acquire the disease and many of them fall a prey to the organism which they carried for a long time in their yolk. Those which survive often become permanent bacillus carriers, and thus the cycle of infection is completed. Furthermore, these chicks are a constant source of infection to other chicks and mature stock as well.

The occurrence of *Bacterium pullorum* in eggs has been a subject of serious consideration, but only from the standpoint of breeding and the perpetuation of sound stock. The system of testing breeding hens by the agglutination test, which is now being conducted in several of the states, marks but a culmination of the efforts that are being made to combat the disease through the detection and the elimination of the permanent carriers of bacillary white diarrhea.

There has been much speculation from time to time as to whether the presence of *Bacterium pullorum* in an egg renders it unsafe as an article of diet for man or any of the lower animals other than chicks, and especially for young children and infants. Little serious thought was given to this question, however, and it was dismissed with the assumption that such an element of danger, if it existed at all, was but very slight. While it is not the purpose of this paper to spread undue alarm, there is sufficient evidence on hand to show that *Bacterium pullorum*, when fed to some of the lower animals, may produce most serious consequences, and even death. In other words, it has been demonstrated that this organism may manifest itself as one of the group of so called "food-poisoning bacteria."

The marked pathogenicity of *Bacterium pullorum* for young chicks has been so frequently demonstrated that little need be said concerning it here. Chicks under 1 week of age are extremely susceptible to the influence of this organism, whether infection takes place through the mouth or the skin (subcutaneous injection), and the mortality is usually very high. It had long been supposed, however, that the ability of this organism to produce disease did not extend beyond young chicks.

Jones[3] was the first to demonstrate that *Bacterium pullorum* may be pathogenic for fully mature fowls. On a large poultry farm in the state of New York

[3] Jones, F. S., *Jour. Med. Research*, 1912–13, xxvii, 471.

a peculiar epidemic manifested itself in a flock of about 700 adult hens, the resultant mortality being 50. This epidemic was most clearly traced to infected eggs, as the following account will show. There had been no history of bacillary white diarrhea on the farm. A neighbor had been meeting with serious losses, however, and a number of eggs from his fowls were incubated on the farm which had hitherto been free from the disease. Nearly all the chicks that were hatched from these eggs died within 10 days from bacillary white diarrhea. The eggs which failed to hatch were inadvertently fed on Mar. 12 to the adult hens. On Mar. 28 a few of the fowls which had eaten the eggs died, and from that time on for a period of a month 50 of the hens died, with essentially the same symptoms. *Bacterium pullorum* was recovered from various internal organs of hens that were examined, as well as from chicks hatched from the neighbor's eggs, and from the ovaries of the neighbor's adult fowls.

We have obtained similar results, though on a much smaller scale. In a series of experiments conducted in 1913 on mature fowls, and in which bouillon cultures of *Bacterium pullorum* were fed to the fowls along with the regular dry mash, three deaths occurred in a pen of twelve hens. Two of the hens were sent to the laboratory for bacteriological examinations. From the liver, lungs, spleen, and heart of both these victims *Bacterium pullorum* was easily recovered, and the growths obtained on agar by direct inoculation were such as to indicate that the bacilli were present in the blood in very large numbers. Postmortem examinations did not reveal any marked or gross lesions. The deaths occurred within a period of 1 to 2 weeks after the first ingestion of the bouillon cultures. Other hens which appeared to be affected completely recovered in a relatively short time.

The first information as to the real toxicity of *Bacterium pullorum* for experimental animals was conveyed by Smith and Ten Broeck.[4] In comparative studies of the properties of this organism and of the bacillus of fowl typhoid these authors demonstrated that the bacterium-free filtrates of 5 to 15 day old bouillon cultures of *Bacterium pullorum* were decidedly toxic to full grown rabbits when given by intravenous injection. Death followed within 2 hours, or there was marked dyspnea followed by death over night or by loss of weight and subsequent recovery.

Gage[5] has shown that rabbits are very susceptible to even very small doses of *Bacterium pullorum* by subcutaneous injection, and that for this reason it is difficult to immunize rabbits to the organism.

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[4] Smith, T., and Ten Broeck, C., *Jour. Med. Research*, 1915, xxxi, 547.

[5] Gage, personal communication.

The present investigation divides itself into two distinct phases: first, a study of the toxicity or disease-producing properties of *Bacterium pullorum* when administered orally, either with food or with the aid of a pipette; and, second, an investigation of the heat tolerance of this organism in infected eggs which are being prepared for table use by the usual processes of boiling, coddling, frying, etc.

The Toxicity of Bacterium pullorum when Introduced through the Mouth and Digestive Tract.

The experiments were conducted on 22 rabbits, 11 kittens, 11 guinea pigs, and 4 white rats. The method of administering the test organism varied. In some instances water suspensions of slant agar cultures were mixed with the regular feed, while at other times these suspensions were given with a pipette. In a number of the experiments the yolks of eggs were employed which had been artificially infected with pure cultures of the organism by injecting relatively small numbers directly into the yolk, through the shell and white, and incubated for at least 2 or 3 days. The different animals were weighed at frequent intervals, usually each day, and their general condition was observed. Those that died were subjected to post-mortem examination, with a special effort to determine the presence or absence of *Bacterium pullorum* in the blood of the internal organs.

In Table I are given the results of the infection experiments with rabbits, in so far as the weights and deaths are concerned. It will be seen that the initial weights of the animals varied from 375 to 2,455 gm., the majority of the rabbits being half grown or smaller. More complete data are given in the individual records following the table.

Rabbits 1 and 2 were fed large amounts of suspensions of five different strains of *Bacterium pullorum* (1 to 5 cc.) daily. On the 5th day they refused to eat, and died on the 7th and 6th days. Pure cultures of *Bacterium pullorum* were obtained from the internal organs. Rabbits 3, 4, 5, and 6 received one large dose of the organism with the food. From the blood of each of these animals the bacterium in question was recovered in large numbers, after death. Rabbits 7 and 8 were given the surface growth of one tube of slant agar. Blood

TABLE I.

Rabbits. Weight in Gm., and Mortality Records.

	Rabbit No.	Initial weight.	Days after first administration of *Bacterium pullorum*.											
			1	2	3	4	5	6	7	8	9	10	40	
1	Infected	380							Dead.					
2	"	375						Dead.						
3	"	650			640		550	540	"					
4	"	420	412	405	417	435	411	387	"					
5	"	475	482	483	488	452	438	Dead.						
6	"	385			380		342	337	"					
7	"	545	558	560	540	500		495	"					
8	"	435	434	432	423	405	Dead.							
9	"	615	592	630	635			550	"					
10	"	745	700	723	730			785		850		840	1,428	Alive.
11	"	648	665	690	685			675		725		770	1,334	"
12	Control	410	412	425	420			407		480		475	1,140	"
13	Infected	2,455	2,430	2,440		2,436			2,485	After 18 days			2,390	"
14	"	885	870	925	1,010	955	1,005		1,060		1,080	After 21 days	1,120	"
15	"	1,240	1,220	1,250	1,360	1,290	1,320		1,365		1,380	" 21 "	1,350	"
16	"	1,100	1,138	1,160	1,250	1,190	1,240	1,075	Dead.					
17	"	985	952	940	805	730	735	Dead.						
18	"	1,180	1,162	1,140	1,300			1,030	"					
19	"	1,370	1,362	1,370	1,325	1,370	1,335	1,215	"					
20	Control	1,308		1,325	1,400	1,310	1,325			1,400	Alive.			
21	"	1,210		1,230	1,285	1,240	1,255			1,290	"			
22	Infected	1,980	1,835	1,845		1,770	1,720	Dead.						

tests were again positive. Rabbit 9 was fed 0.1 of a slant agar growth. Death occurred on the 7th day. *Bacterium pullorum* was demonstrated in the blood.

Rabbit 10 received 0.3 of a 24 hour slant agar culture. It continued to gain weight for 40 days. It was then given 0.5 cc. of a mixture of three strains, but continued to gain for 2 weeks. A third dose of 0.7 cc. likewise had no visible effect.

Rabbit 12 served as a control animal, and was therefore not subjected to an infection experiment. Aside from this rabbit there were at all times at least two or three rabbits which belonged to the same lots as the test animals, and which were kept under practically the same conditions, except for the administration of suspensions of *Bacterium pullorum*. None of these stock rabbits died or showed any indications of illness.

Aside from a loss of 65 gm., Rabbit 13 was apparently unaffected after receiving through a pipette 2 cc. of a suspension obtained by washing three slant agar cultures.

Rabbits 14 and 15 received 0.5 and 1.0 cc. respectively of the yolk of incubated eggs which had been artificially infected with *Bacterium pullorum*. Rabbits 16, 17, 18, and 19 were given with a pipette 1.5, 2.0, 3.0, and 5.0 cc. of the yolk of artificially infected eggs. In addition to losses in weight, and death in each instance, there were indications of a diarrheal condition. With the exception of No. 18, blood tests with each of these animals for *Bacterium pullorum* gave positive results.

Rabbits 20 and 21 were kept as controls in separate cages. They remained apparently normal.

Rabbit 22 was a good sized adult male which had been suffering from a large abdominal abscess. Although the abscess was lanced it continued visibly to affect the health of the animal. 10 cc. of the yolk of an infected egg were given by mouth. On the 5th day there were marked diarrheal symptoms, and on the 6th day the rabbit died. *Bacterium pullorum* was recovered from the heart, liver, and lungs in large numbers.

Postmortem examination of the rabbits which died apparently from the effects of infection with *Bacterium pullorum* revealed no gross

lesions or other marked pathological condition. The small intestine was usually empty and decidedly pale. At times a light yellow viscid fluid was present in the lumen. In the lower intestine the contents were often less firm than in the normal rabbit, and frequently there was evidence of a diarrheal condition.

The liver was more or less congested. The surface was often marked by minute areas which appeared to be of a necrotic character. Aside from some congestion the spleen and kidneys were to all appearances normal. No pathological condition of the heart and lungs was visible to the unaided eye. Since no investigation was made of the histological structures of the different organs no details can be given as to their minute pathology.

The internal organs of the cats that died showed no marked pathological appearances, and to a large extent resembled those of the rabbits that succumbed to infection. Congestion was apparent, especially in the liver. Culture tests upon Cats 1, 2, 6, 7, and 8 gave positive results, *Bacterium pullorum* being recovered with ease from the blood of the liver, kidney, and lungs, except in No. 7 in which only the lungs contained the organism in question in sufficient numbers to obtain positive cultures. Agglutination tests with positive fowl sera proved the organism to be *Bacterium pullorum*.

The early death of the control animal was either due to some other cause or agent, aside from *Bacterium pullorum*, or was the result of rapid infection of this kitten from the vomited matter and diarrheal discharges of other kittens that were the first to be seriously affected. These experiments on kittens are to be repeated, with enough controls kept in separate cages to make the results as conclusive as possible.

White Rats.—Four white rats weighing from 100 to 250 gm. were used in these experiments. They were given one large dose of *Bacterium pullorum* in a water suspension. None of the animals showed any signs of discomfort or illness after the treatment, and all continued to increase in weight for the entire observation period of 24 days. The rats, although small as compared with adults, were apparently unaffected by one treatment with the organism (3 to 5 cc. of the suspension).

TABLE II.

Cats. Weight in Gm., and Mortality Records.

Cat No.	Initial weight.	Days after first administration of *Bacterium pullorum* by mouth. 1	2	3	4	5	6	8	10	12	14	20	23	40
1	1,088	—	1,180	1,160	—	1,192	—	1,115	1,074	985	915	790	Dead.	
	Given 2.0 cc. of suspension of *Bacterum pullorum* (with pipette) at beginning of experiment.													
2	1,098	—	1,158	1,136	—	1,200	—	1,130	997	955	890	750	"	
	Given 1.0 cc. of suspension.													
3	928	—	940	920	—	846	—	805	776	755	750	810	795	Dead. 635
	Given 0.5 cc. of suspension.													
4	618	—	632	—	—	535	492	Dead.						
	Received 0.5 cc. of suspension of *Bacterium pullorum* in water.													
5	600	—	600	—	—	590	636	535	525	530	Dead.			
	Given 1.0 cc. of suspension.													
6	590	—	590	—	—	485	492	Dead.						
	Given 1.5 cc. of suspension.													
7	660	—	680	—	—	615	620	575	595	Dead.				
	Given 2.0 cc. of suspension.													
8	620	—	820	—	—	800	710	Dead.						
	Control. Not artificially infected, but kept in same enclosure as Cats 4 to 7.													
9	990	935	910	895	885	880	—	795	770	770	685	700	710	Alive.
	Received 0.5 cc. of suspension.													
10	990	1,000	965	980	980	940	—	990	925	905	865	880	890	"
	Given 1.0 cc. of suspension.													
11	730	740	746	725	745	705	—	710	685	695	690	650	Alive.	
	Given 0.5 cc. of suspension.													

Kittens 1 to 3 were from one litter, Nos. 4 to 8 from another, and Nos. 10 and 11 from a third. While No. 8 was not artificially infected she was allowed to be in contact with Nos. 1 to 7 which were fed suspensions of *Bacterium pullorum* with a pipette. As vomiting and purging occurred very soon in these cats, the possibility of the control's becoming infected early cannot be excluded.

Kittens 4 to 7 were fed the bacterial suspensions 3 days later than Nos. 1 to 3. Signs of disturbances became apparent in both groups at about the same time. Vomiting, diarrhea, and rapid loss in weight were the most marked symptoms in the animals that died. Vomiting and diarrhea were most acute. The temperature during the time of the most severe attacks was decidedly subnormal, and for several days before death the kittens were extremely weak and listless. Cats 9, 10, and 11 showed no signs of illness except loss in weight.

TABLE III.

Guinea Pigs. Weight in Gm., and Mortality Records.

Guinea pig No.	Initial weight.	Days after first administration of *Bacterium pullorum* by mouth, and of additional doses. 1	2	3	4	5	6	8	10	12	14	16	18	20	
1	540	—	—	560	—	540	525	528	529	525	515	510	510	538	Alive.
	Bacterial suspension of *Bacterium pullorum* mixed with food at beginning of experiment.														
2	510	—	—	512	—	502	495	482	512	498	505	518	514	555	"
	Same treatment as No. 1.														
3	717	718	710	710	700	723	702	703	—	694	710	698	705	740	"
	Same treatment as No. 1.														
4	365	370	385	—	360	340	325	Dead.							
	Given varying amounts of infected yolk daily for 6 days (1 to 5 cc. of yolk.)														
5	75	78	80	85	84	—	83	Dead.							
	Given 0.5 cc. of heavy bacterial suspension at beginning of experiment.														
6	365	370	385	360	340	325	330	275	Dead.						
	3cc.	3cc.	5cc.	8cc.	—	3cc.	8cc.	of infected yolk, given on respective dates.							
7	425	425	420	405	405	410	400	415	430	—	—	—	—	Dead.	
	7cc.	5cc.	6cc.	5cc.	4cc.	of infected yolk.									
8	315	—	310	—	330	315	—	330	—	335	—	—	—	355	"
	10cc.				10cc. of infected yolk.										
9	315	—	310	—	265	285	—	295	—	300	—	—	—	325	"
	10cc.				10cc. of infected yolk.										
10	345	320	Dead.												
	One feeding, half of an infected yolk.														
11	405	380	355	320	Dead.										
	One feeding, half of an infected yolk.														

In Guinea Pig 4 a diarrheal condition was apparent on the last 2 days. The liver and kidneys were congested. Other organs were normal. Cultural tests were negative. Guinea Pig 7 showed evidence of marked diarrhea just before death. There was some congestion of the liver and spleen, and hemorrhage in the pericardial sac. Culture tests from the different organs were negative. In Nos. 6, 7, and 10 slight diarrhea was apparent, but no other symptoms, except loss of weight and lessened appetite, were observed.

Cultural tests were again negative. *Bacterium pullorum* was isolated from the liver and lungs of Guinea Pig 11, but not from the blood of any of the other five animals that died.

DISCUSSION.

The foregoing data on the oral administration of *Bacterium pullorum* can leave no doubt as to the so called toxicity of this organism for young rabbits when given by mouth. The results of the feeding experiments with kittens of different ages are less conclusive, and require further substantiation, in view of the fact that there was no satisfactory control. They are strongly indicative, however, of a most harmful influence exerted by relatively large numbers of the organism when administered by mouth. The symptoms of the disturbance produced in the kittens were those of a food-poisoning organism, especially the vomiting and diarrhea, and the extreme emaciation. The possibility that these symptoms are due to a complication of disturbing factors, cannot be entirely ignored, however.

Adult guinea pigs are but slightly susceptible to *Bacterium pullorum*, when ingested, though six of the eleven animals employed died, some early and others late in the periods of observation. With but one or two exceptions, they were given large amounts of the organism. Further experiments with guinea pigs are now in progress. Rats, according to the above data, are immune to disturbing influences of *Bacterium pullorum* when it is given by the mouth, even in large numbers.

Although there are no cases on record of food-poisoning, enteritis, or other ailments in man which have been ascribed to *Bacterium pullorum*, the possibility of danger from infection with this organism can no longer be ignored, especially in so far as invalids and young children are concerned. Eggs are among the most common articles of diet, and for the sick and convalescent as well as for children of all ages they often rank with milk as one of the most important foods. Not only are they consumed soft boiled, but they are frequently prescribed in the raw state.

Fresh eggs, when they are infected with *Bacterium pullorum*, contain this organism in such small numbers as to constitute no real danger of disturbance even for the most infirm, or for the smallest infants. It has been conclusively demonstrated, however, that the organism multiplies very rapidly in the yolks of infected eggs, when

the eggs are held at or near so called incubator temperature.[6] Hence, eggs which are left in the nest under broody hens for but comparatively few hours, or which are not kept in cool places during storage and transportation, especially during the warm summer months, harbor the organism in large numbers. In fact, they are so abundant that the inoculation of the surface of slant agar with but a small portion of the yolk held in a platinum loop results in the production of an almost solid surface growth on the agar.

It has also been shown that in various sections of this country a large percentage of the flocks and of the individual hens are permanent carriers of white diarrhea (*Bacterium pullorum*) infection. In the survey of the conditions in Connecticut it was found that out of 107 flocks which were tested for ovarian infection with *Bacterium pullorum* 79, or 74 per cent, possessed bacillus carriers, and of a total of 13,831 fowls that were subjected to the agglutination test 1,417, or 10.24 per cent, gave positive indications of ovarian infection.[7] In an investigation recently conducted in Massachusetts similar results were obtained.[8] In some flocks over 50 per cent of the individual fowls that were tested were reactors. The same condition undoubtedly prevails throughout a large part of the Country, though no definite figures have been obtained outside of Connecticut and Massachusetts.

The common methods of boiling or frying infected eggs for the table do not necessarily render the eggs sterile, in so far as *Bacterium pullorum* is concerned, as the following data will show.

The Survival of Bacterium pullorum in the Yolks of Eggs after Various Methods of Treatment with Heat. The Influence of Cooking.

These experiments were carried on with eggs which were artificially infected with *Bacterium pullorum* in the following manner. Fresh

[6] Rettger and Stoneburn, *Bull. Storrs Agricultural Experiment Station*, 1911, No. 68. Rettger, Kirkpatrick, and Jones, *Bull. Storrs Agricultural Experiment Station*, 1914, No. 77. Rettger, *Centralbl. f. Bakteriol., 2te Abt.*, 1913–14, xxxix, 611.

[7] Rettger, Kirkpatrick, and Jones, *Bull. Storrs Agricultural Experiment Station*, in press.

[8] Gage, G. E., and Paige, B. H., *Bull. Massachusetts Agricultural Experiment Station*, 1915, No. 163.

eggs were immersed for a few minutes in alcohol, and one of the ends was flamed. A small hole was made through the shell without injuring the shell membrane. The eggs were then inoculated by injecting about 0.25 cc. of a water suspension of the organism with a sterile hypodermic syringe directly into the yolk. The hole was sealed with collodion and the eggs were incubated for varying lengths of time, usually 3 to 5 days.

Infected eggs were placed in boiling water, and held there for different lengths of time. They were then chilled in cold water and opened aseptically. Small amounts of yolk were streaked over the surface of slant agar and the tubes incubated at 37°C. The results are given in Table IV.

TABLE IV.

Time of boiling.	Coagulation of white.	Thickening of yolk.	Growths of *Bacterium pullorum* on slant agar.
min.			
1.....	0	0	+
2.....	0	0	+
2½.....	Slight.	0	0 + + + + +
3.....	+	Slight.	0 0 0 + + + +
3½.....	+	+	0 0 0 0 0 0 + + + +
4.....	+	+	0 0 0 + +

In the last column 0 indicates no growth, and + the characteristic growth of *Bacterium pullorum*. Each 0 and + sign represents an individual egg. The variation in the results of the different eggs is undoubtedly due to differences in the size of the eggs and the thickness of the shells, and perhaps in part to the differences in the numbers of bacteria in the eggs.

Poaching the eggs for ½ to 4 minutes rendered them sterile. Artificially infected eggs that were scrambled were likewise found to contain no viable organisms. On the other hand, fried and coddled eggs gave varied results, as will be seen in the following brief summaries.

In all the foregoing experiments control tests were made with eggs that had been inoculated and incubated in the same way as the others. No difficulty was experienced in obtaining an abundance of the bacilli in the yolks after the various periods of incubation of the eggs.

It should be stated that all these experiments were conducted at an altitude of 700 feet above sea level, where the boiling point of water is 99.2°C.

TABLE V.

The Effect of Frying.

Physical character of heated eggs.	Growth on slant agar.
Frying on one side only.	
Soft.	+ + + +
Medium.	+ +
Hard.	+
Frying on both sides.	
Soft.	0 0
Medium.	0 0
Hard.	0 0

TABLE VI.

The Effect of Coddling.

Time of heating.	Growth on slant agar.
min.	
3	0 +
$3\frac{1}{2}$	0 0
4	0 + + +
$4\frac{1}{2}$	+
5	0 0
7	0
10	0

The eggs were coddled by pouring boiling water over them in a granite receptacle and allowing them to stand away from a flame or stove for the different lengths of time. About 1 quart of water was employed for each egg.

The experiments on the viability of *Bacterium pullorum* in egg yolk after varying periods of heating show that the organism under the stated conditions possesses a high degree of resistance. Even boil-

ing for 4 minutes did not in every instance destroy it. This resistance is due undoubtedly to the peculiar protection which is afforded, first by the shell, then by the egg white, and finally by the yolk itself. The high per cent of fat in the yolk is undoubtedly an important factor.

It has been demonstrated by Smith[9] and others that tubercle bacilli are less readily killed by the ordinary process of pasteurization when they are held in the film or pellicle layer of milk than in the whole or mixed milk.

Other organisms of the colon-paratyphoid-typhoid group would undoubtedly show the same resistance under similar conditions of environment. Chantemesse and Rodriguez[10] report an epidemic of food-poisoning which was due to cream cakes. The meringue of the cakes was found to contain a peculiarly toxic microorganism of the *Bacillus paratyphi* type, and although the meringue had been heated to browning numerous bacilli of this type were found in the interior portion. Quite recently a small epidemic of typhoid fever was pronounced to have been caused by the consumption of baked spaghetti.[11] The typhoid bacillus was readily identified in the inner portion of spaghetti that was artificially inoculated and baked, in spite of the fact that the dish was subjected to sufficient heat to brown the surface and to char the protruding ends of the spaghetti sticks.

SUMMARY AND CONCLUSIONS.

The problem of eradicating ovarian infection in the domestic fowl assumes still greater importance than heretofore, in the light of data recently acquired. Not only is it of great significance to eliminate the permanent carriers of *Bacterium pullorum* from all flocks of fowls from the standpoint of successful poultry breeding, but also because they constitute a possible source of danger to man.

Eggs which harbor *Bacterium pullorum* in the yolk in large numbers may produce abnormal conditions, when fed, not only in young chicks, but in adult fowls, young rabbits, guinea pigs, and kittens. The toxicity for young rabbits is most pronounced, the infection usually resulting in the death of the animals. In kittens the most prominent symptoms are those of severe food-poisoning with members of the paratyphoid group of bacteria. The possibility of infected eggs causing serious disturbances in young children and in the sick

[9] Smith, T., *Jour. Exper. Med.*, 1899, iv, 217.

[10] Chantemesse and Rodriguez, *Bull. Acad. de méd.*, 1914, lxxi, 245; Abstract in *Experiment Station Record*, 1914, xxxi, 555.

[11] Johnston, H., *Health News*, 1915, xxxi, 173.

and convalescent of all ages must therefore receive serious consideration.

Ovarian infection of fowls is very common throughout this country. Hence, a large proportion of the marketed eggs are infected with *Bacterium pullorum*. When such eggs are allowed to remain in nests under broody hens, or in warm storage places, for comparatively few hours, they contain large numbers of the organism.

Soft boiling, coddling, and frying on one side only do not necessarily render the yolks free from viable bacteria; therefore, eggs which have gone through these processes may, like raw eggs, be the cause of serious disturbances in persons who are particularly susceptible to such influences, and especially to infants.

That no well authenticated instances of egg-poisoning of this kind are on record does not warrant the assumption that there have been no cases. The etiology of infantile stomach and intestinal disturbances is as yet too little understood; in fact, it may be said that many of these disorders have no known cause, and almost as much may be said regarding gastro-intestinal diseases in later life. Furthermore, since the ailments caused by infected eggs would not make themselves felt presumably until several days after their ingestion, little or no suspicion would fall upon the eggs. It may be said, too, that the wide distribution of ovarian infection in the domestic fowl has come about only in the last few years, hence its possible danger to man is one of recent development.

SERUM CHANGES AND THE CAUSE OF DEATH IN EXPERIMENTAL PANCREATITIS.

Studies on Ferment Action. XXX.

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(Received for publication, January 26, 1916.)

Incidental to a study of the ferment balance of the serum during various pathological conditions we have had occasion to observe the serum changes in a series of eighteen dogs in which an acute experimental pancreatitis had been produced.

Various theories have been advanced to account for the marked intoxication resulting from an acute pancreatitis. Opie (1) in this country, and Eppinger (2), Pólya (3), von Bergmann and Guleke (4), and numerous other workers in Europe have fully discussed the problem. The changes in the lipase titer have been reported by Whipple and Goodpasture (5).

Most workers are convinced that an activation (probably intracellular) of the tryptic proenzyme occurs with a resulting intoxication of the animal because of the sudden formation and absorption of toxic split products. Coincident with this change there may occur a saponification of fats due to the simultaneous activation of the pancreatic lipase with a further injury to the pancreatic tissue by the soaps so formed. The means of activation of these ferments, resulting from a primary injury, may, of course, be diverse: bacterial infection, either through the ducts or lymphatics; mechanical blocking of the ducts and activation from bile; or activation from enterokinase and from tissue juice resulting from direct trauma, possibly from injury following vascular changes. While, therefore, the primary factor may be diverse, the resulting pathological lesions and the cause of death are uniform.

In the following illustrative experiments three different substances were injected as activators: bile salts, as first recommended by Flexner (6), active trypsin solutions, and sodium oleate solutions.

These experiments and the charts of the serum changes follow.

Dog 1.—Weight 4.5 kilos. 0.2 gm. of bile salts was injected into the pancreatic duct with subsequent ligation, at 9.30 a.m. on May 10, 1915. Serum samples were collected before the operation, at 10 and 11 a.m., and at 1 and 3 p.m. on each of the following 2 days and after about 3 weeks (June 3, 1915). The serum changes are shown in detail in Text-fig. 1.

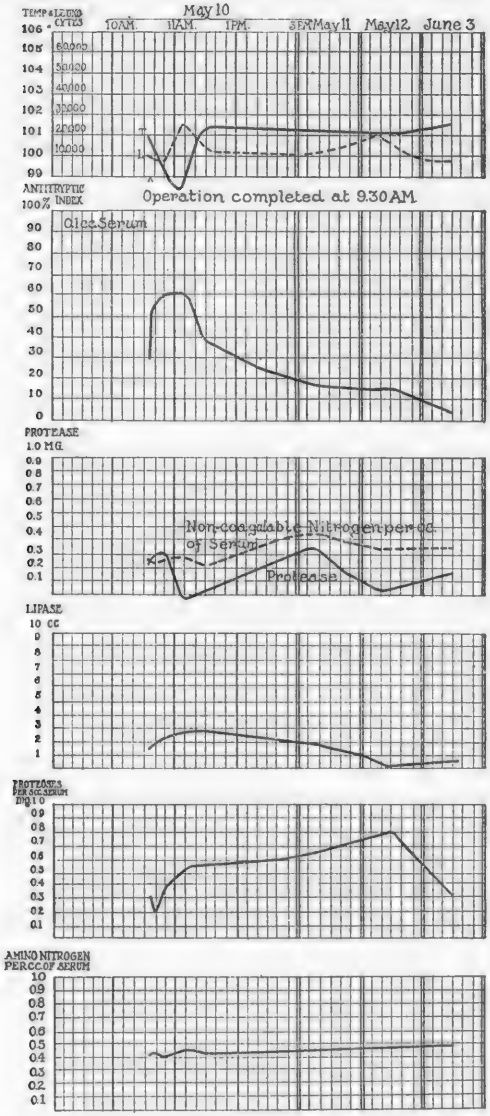
The animal was killed on June 3, 1915. The pathological changes noted at autopsy were as follows: The pancreatic duct was occluded. The body of the pancreas was atrophic, fibrous, with large areas of fatty change and small necrotic foci containing caseous material. The tail of the pancreas showed less change. The viscera presented no pathological alteration other than fatty changes.

Dog 2.—Weight 5 kilos. 1 gm. of sodium oleate was injected into the pancreatic duct with subsequent ligation, at 11.30 a.m. (May 14, 1915). Serum samples were collected before the operation at 11.50 a.m., and at 1.30 and 3.30 p.m. on the 3 succeeding days, and after several weeks (June 4, 1915). The dog was killed after being used in another experiment. The serum changes are shown in detail in Text-fig. 2. The findings at autopsy were as follows: The pancreatic tissue was represented by a small remnant about one-fourth the size of the original tissue, rather firm, and pale yellow in color. The duct was occluded and there were numerous firm adhesions to the adjoining viscera. Scattered throughout the remnants of the gland were small more or less caseous areas of necrosis.

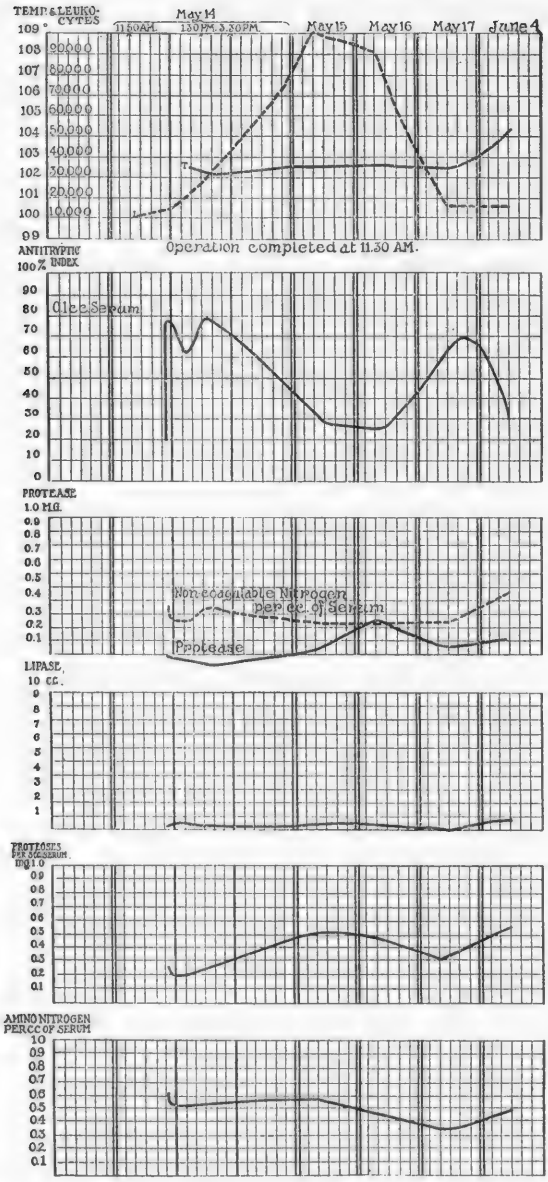
Dog 3.—Weight 5.1 kilos. 0.2 gm. of trypsin (purified) was injected into the pancreatic duct, with subsequent ligation, at 9.10 a.m. (June 7, 1915). Serum samples were collected before the operation at 9.45 a.m., noon, and 3 p.m., and at 8.30 a.m. and 2 p.m. the following day. The animal died at 2.15 p.m. (June 8, 1915). The serum changes are shown in detail in Text-fig. 3.

The autopsy findings were as follows: On opening the abdominal cavity a considerable amount of sterile, hemorrhagic, opaque fluid was found. This exudate contained 0.28 mg. of non-coagulable nitrogen per cc., and had a slight antitryptic action. Incubated under toluol the non-coagulable nitrogen increased 0.27 mg., and under chloroform 1.72 mg. in 16 hours, indicating the presence of considerable amounts of proteolytic ferments. The pancreatic tissue was hemorrhagic and contained numerous areas of necrosis throughout; there were numerous areas of fat necrosis scattered over the omentum. Fibrinous adhesions were found about the pancreas and adjacent viscera.

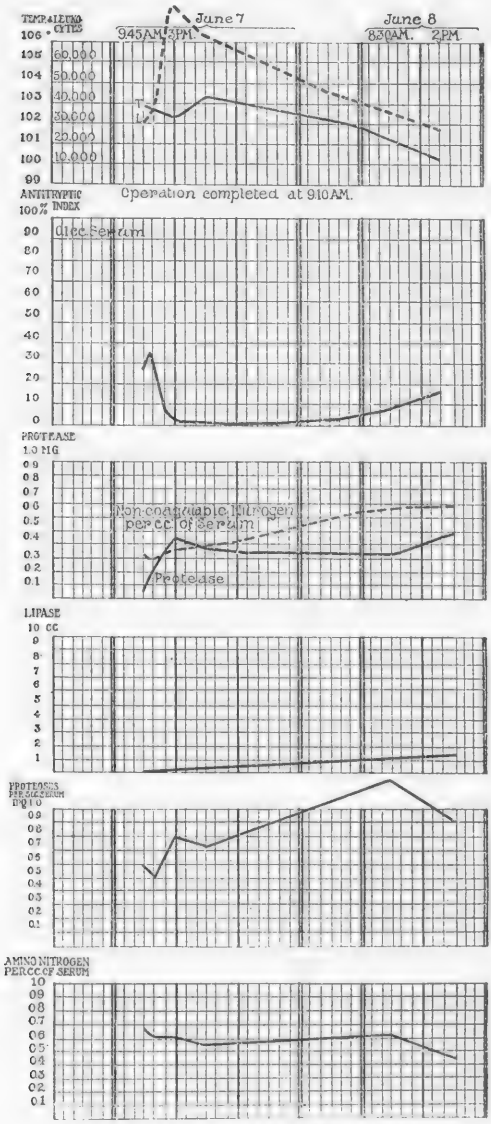
In the first animal the injection of bile salts caused only a moderate pancreatitis, although at the time the abdomen was closed after the injection a thorough infiltration of the pancreatic tissue was noted.



TEXT-FIG. 1. Serum changes accompanying acute pancreatitis due to bile salt injections.



TEXT-FIG. 2. Serum changes accompanying acute pancreatitis due to soap injections.



TEXT-FIG. 3. Serum changes accompanying acute pancreatitis due to trypsin injections.

No increase in temperature resulted; only a moderate leukocytosis and that only for a short period of time. Immediately after the operation the antiferment increased while the protease decreased; the balance was restored to a normal level the following day. The serum lipase increased slightly. There was noted a marked increase in serum proteoses during the period following the operation; after 3 weeks a normal value was again found. The amino nitrogen in this, as in the other animals, showed only very slight changes.

In the third dog the injection of the trypsin resulted in a rapidly fatal intoxication and it will be observed that the serum changes were different. There was a marked leukocytosis—80,000—as an index of the intoxication, with a progressive decrease in temperature. The antiferment, after a short rise, declined progressively, while the serum protease increased and remained high. The proteoses, after a slight initial decrease, frequently observed in the period following the mobilization of protease, increased markedly. There was only a slight change in the lipase.

The picture closely resembles the condition found in true trypsin shock (7), except in the behavior of the serum lipase. In conjunction with Experiment 2 it would seem that the decrease in the antiferment and the increase in protease have had some relation to the fatal outcome in this case.

In the second dog in which soap was injected, we find the reverse of the preceding changes, as might be expected from the difference in the action of the substances. The tissue destruction was quite marked and the whole pancreas even before closing the abdomen presented a deeply engorged, hemorrhagic, semitranslucent mass almost twice the normal size. It will be observed that the leukocyte count was also high—100,000 after 24 hours. Despite the severity of the local process the serum changes were slight and the animal showed little evidence of a general intoxication. The antiferment increased immediately, but the serum picture was practically unaltered after 24 hours. The lipase remained low. As contrasted with the two previous animals it will be seen that the increase in proteoses was not only delayed but actually less in amount.

DISCUSSION.

From the series of animals, of which these three are representative, we believe that we are justified in assuming that death is due to the sudden flooding of the blood stream with the higher split products formed at the expense of the pancreatic tissue, of which the proteose increase is an index. Except in the experiments in which active trypsin is used for injection there is no increase in serum protease at any time, as would be expected if the intoxication were a true trypsin shock, nor is there much change in serum lipase (esterase), the condition in this respect resembling closely the results observed following the injection of protein split products (8).

Here, as in other protein intoxications (9) the increase in antiferment seems to be of distinct value in the protection of the animal; in the experiment in which soap was used for the injection this becomes apparent. The increase in antiferment was marked, while the delay in the digestion of the pancreas and the consequent lessening of the shock is indicated by the gradual and relatively small increase in the amount of proteoses present in the serum.

In view of Whipple's results concerning the non-toxicity of the abdominal exudate (5) and our own experiments, it would seem that the only beneficial effects which might possibly have accrued from operative interference in cases of human pancreatitis have resulted from the prolonged anesthesia rather than from the surgical drainage. The inhibitory effect of anesthetics on the development of toxicity for protein intoxication is well known.

CONCLUSIONS.

1. The serum changes observed during acute experimental pancreatitis indicate that the shock and death are due to an intoxication from protein split products, and not to an intoxication from pure tryptic ferment.

2. When the pancreatitis is produced by the injection of an anti-proteolytic substance (sodium oleate), the degree of intoxication bears no relation to the degree of tissue destruction.

3. The increase in serum antiferment apparently favors the recovery of the animal.

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SERUM CHANGES FOLLOWING THYROPARATHYROIDECTOMY.

STUDIES ON FERMENT ACTION. XXXI.

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As a result of the work of MacCallum and his coworkers (1), our knowledge concerning disturbances of the inorganic constituents of the blood serum particularly, following the removal of the parathyroids has been greatly advanced, and the antagonistic effect of the calcium ions established.

Certain observations have accumulated, largely of a clinical nature, which seem to indicate that in the tetany of infancy dietary factors are of considerable importance both from an etiological and therapeutic point of view. The spasmophilic infant rapidly recovers on a proper diet, and the evidence seems to indicate that phosphorus and certain of the unsaturated oils are of aid in expediting a favorable outcome.

In adults, too, we have to consider the peculiar form of tetany associated with gastric and occasionally intestinal disturbances, under conditions, therefore, which may be considered to offer opportunities for the absorption of the higher split products of proteins, the gastric digestion, of course, only splitting through to the peptones.

Experimentally the observation has been frequently recorded that the feeding of meats is particularly prone to lead to the onset of tetany in parathyroidectomized dogs.

Considerations such as these probably led Kling (2) to study the electric excitability of animals during anaphylaxis, in an effort to correlate the increased irritability of tetany with an anaphylactic state depending on a protein intoxication.

Our knowledge concerning the pathological effects of a faulty absorption of the higher split products of proteins from the gastro-intestinal tract is as yet fragmentary, despite the obvious clinical evidence pointing in this direction. It has been observed, however, that in gastric tetany a gastro-enterostomy usually relieves the condition, the operative interference probably enabling a more rapid emptying of the stomach to the intestine with an augmented splitting of the protein contents to lower stages. Kaufmann's work is of particular interest in this connection (3).

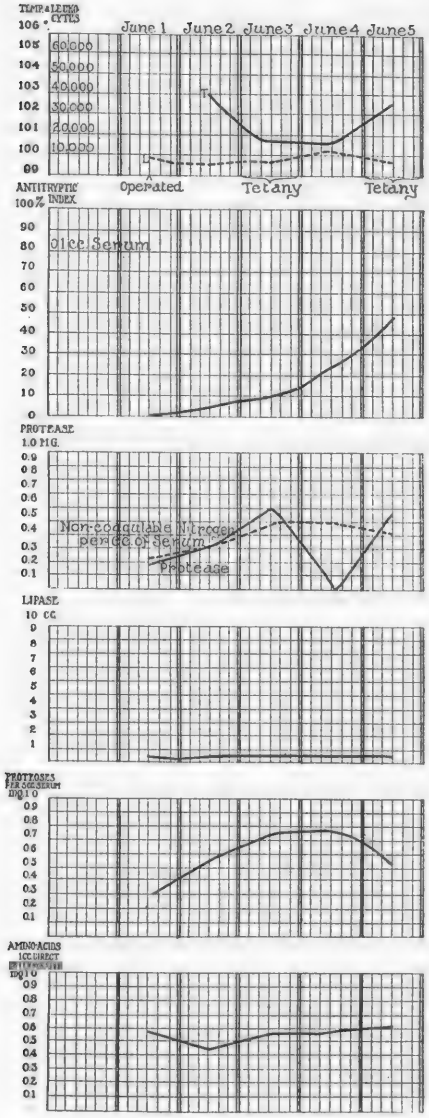
In the few cases of true intestinal tetany that have been reported, there is considerable evidence that an achylia gastrica frequently complicates the picture, which again would offer every opportunity for a rapid absorption of the higher split products from the intestine, which under normal conditions is only afforded an opportunity to absorb the lower split products (Fleiner (4), Quosig (5)). The relation of tetany in infants is of particular interest in this connection; for substances which tend to increase the antiferment titer—unsaturated fats, phosphorus—have on empiric grounds been employed in the therapy of spasmophilia for many years, indicating that a suppression of a possibly faulty splitting of proteins may form the basis for this theory. Our interest in experimental tetany has centered chiefly, therefore, in a study of the changes of the serum and the relative amounts of split products therein contained.

The experiments were made on dogs. Thyroparathyroidectomy was performed in fifteen animals, and the serum changes were studied. The following two experiments are typical of the relations noted.

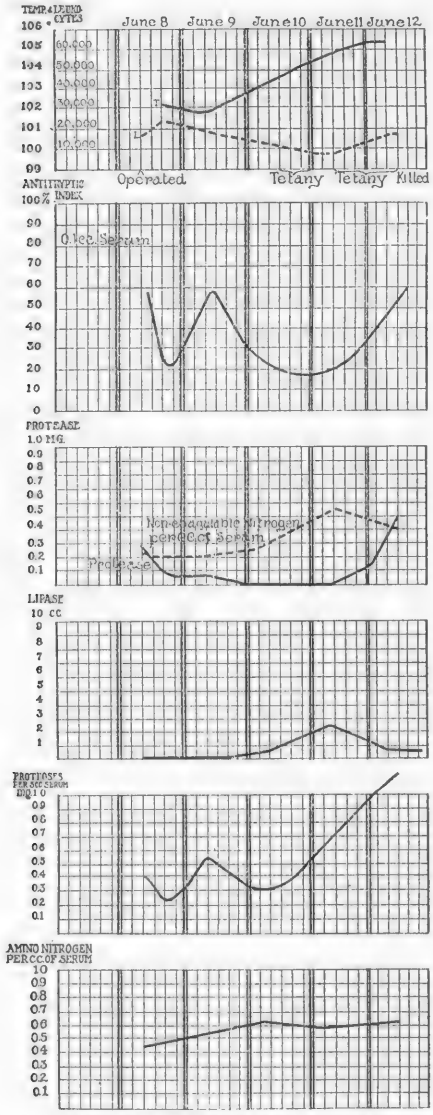
Dog 1.—Weight 5 kilos. Complete thyroparathyroidectomy on June 1, 1915. Tetany was observed on the 3rd day following the operation; on the 4th day the dog showed no symptoms; but on the next day (June 5, 1915) there was marked tetany. The animal was found dead the following morning. In Text-fig. 1 the serum changes are illustrated in detail.

Dog 2.—Weight 8 kilos. Complete thyroparathyroidectomy on June 8, 1915. Tetany was noted in the afternoon of June 10, 1915, again in the following afternoon, and the next morning (June 12, 1915), when the animal was killed. The serum changes are shown in Text-fig. 2.

In the first dog there will be noted a gradual increase in the antiferment titer until the time of death, with an irregular protease curve.



TEXT-FIG. 1. Serum changes following thyroparathyroidectomy in Dog 1.



TEXT-FIG. 2. Serum changes following thyroparathyroidectomy in Dog. 2.

The maximum protease activity was noted in the animal during the time when the tetany was most apparent. The non-coagulable nitrogen of the serum increased to more than twice the original amount. The lipase remained constantly low. The proteoses increased markedly. The amino nitrogen of the serum in this animal showed no change except an initial decrease.

In Experiment 2 (Dog 2) the conditions are different. The antiferment titer showed marked fluctuation, the first decline appearing shortly after the operation. The protease remained low until the last day, but the non-coagulable nitrogen increased as in the previous animal; the proteoses also accumulated during the period of tetany. The increase in amino-acids is similar to that observed in practically all the other animals during tetany. This is the only animal of the entire series in which a rise in the lipase titer was observed.

DISCUSSION.

It is apparent that the ferment changes in these animals are not in themselves related to the onset of tetany, because the protease and lipase titers, as well as the antiferment index, have no constant relation at any time. It is equally apparent that a considerable accumulation of non-coagulable nitrogen occurs in the serum long before the onset of the actual tetany.

Thus an average of all the dogs examined gives the following figures for the non-coagulable nitrogen per cc. of serum:

Before operation.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
0.21 mg.	0.31 mg.	0.39 mg.	0.50 mg.	0.44 mg.

The average proteose content per 5 cc. of serum was as follows:

Before operation.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
0.41 mg.	0.50 mg.	0.58 mg.	0.74 mg.	0.84 mg.

In four animals of the series the onset and recovery from tetany followed at equal time intervals, and the amino-acid content of the serum per cc. has been averaged under comparable conditions.

Before operation.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
0.48 mg.	0.48 mg.	0.59 mg.	0.54 mg.	0.59 mg.
Operated.	No tetany.	Tetany.	No tetany.	Tetany.

Some observers, among them Morel (6), consider the pathological effect of the parathyroidectomy due to an auto-intoxication, regarding the tetany as a secondary character. An increased nitrogenous metabolism following the removal of the parathyroids is well recognized, but it is difficult to obviate the effect of the tremendous muscular stimulation in the interpretation of the result. It is interesting to note that Falta and Kahn (7) noted an increase in the peptide nitrogen of the urine in their animals, a result possibly of the large increase in the serum which we have observed.

Whether or not the alteration in the nitrogenous constituents indicated by the serum and urine changes is wholly the result of the increased muscular activity due to the increased irritability of the nervous system, or whether the change in these constituents from a quantitative and possibly a qualitative point of view is the cause of the increase of irritability of the nervous system, is, of course, not determined in these experiments.

During the course of the work it was observed that despite the marked fluctuation of the serum protease the titer of the serum lipase remained constantly at a very low level. Stuber and Heim (8) have previously expressed the opinion that the titer of the serum lipase (esterase) was at least partially under the control of the glands of internal secretion. In a series of dogs we have injected dried typhoid bacteria (10 to 20 mg.), which normally cause a prompt and not inconsiderable mobilization of serum lipase, into the circulation of thyroparathyroidectomized dogs and into dogs after complete parathyroid and partial thyroid removal. In the majority of instances the mobilization of lipase has been less constant and of less magnitude than in normal dogs, but the experiments are not sufficiently clear cut to warrant the conclusion of a definite relation between the glandular function and the lipase titer.

CONCLUSIONS.

1. In thyroparathyroidectomized dogs the onset of tetany bears no constant relation to the ferment-antiferment balance of the serum.

2. The serum lipase titer remains at a low level throughout.

3. A progressive increase in non-coagulable nitrogen and proteoses is observed in the serum following the removal of the glands.

4. The amino nitrogen of the serum is usually increased at the time when tetany is most marked.

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THE DISAPPEARANCE OF DEXTROSE FROM THE BLOOD AFTER INTRAVENOUS INJECTION.

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(Received for publication, January 27, 1916.)

HISTORICAL.

Claude Bernard[1] was the first to study the elimination of sugars after intravenous administration. He, as well as others,[2] demonstrated that dextrose, lactose, or saccharose, when thus injected in large quantity, is eliminated in the urine. Although working only qualitatively, he[3] realized that dextrose was better assimilated under these circumstances than saccharose or lactose. The time relations were worked out, particularly by von Becker, who found after injecting 1.5 gm. of dextrose into rabbits that sugar elimination by the kidneys began promptly and continued for 5 or 6 hours. Limpert and Falck[4] did the first quantitative work. Dogs of 5 or 6 kilos when given 5 to 7 gm. of dextrose intravenously excreted only traces in the urine. Larger amounts led to a slightly increased excretion. Thus it was shown early that after intravenous injection fairly large amounts of dextrose can be retained. The proportion excreted in the urine, according to von Brasol[5] and others[6] bears no constant relation to the injected dextrose; nor can the duration of the glycosuria be predicted. Von Brasol also found that the degree and duration of glycosuria were not always the same for

[1] Bernard, C., Dissertation, Neue Funktion der Leber als zuckerbereitendes Organ des Menschen und der Thiere, Würzburg, 1853.

[2] Kersting, *Jour. f. prakt. Chem.*, 1844, xxxiii, 58. Baumert, M., *Jour. f. prakt. Chem.*, 1851, liv, 357. Uhle, Inaugural Dissertation, Leipzig, 1852. von Becker, F. J., *Ztschr. f. wissensch. Zool.*, 1854, v, 123. All quoted by Limpert, L., and Falck, C. P., *Virchows Arch. f. path. Anat.*, 1856, ix, 56.

[3] Bernard, C., *Compt. rend. Acad.*, 1846, xxii, 536.

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[6] Pavy, F. W., *Jour. Physiol.*, 1899, xxiv, 479. Weyert, F., *Arch. f. Physiol.*, 1891, 187. Lilienfeld, C., *Ztschr. f. diätet. u. physik. Therap.*, 1898–99, ii, 209. Wilenko, G. G., *Arch. f. exper. Path. u. Pharm.*, 1911, lxvi, 143. Bang, I., Der Blutzucker, Wiesbaden, 1913, 76.

a given animal. Gilbert and Carnot,[7] however, maintained that within certain limits the proportion of sugar eliminated is constant for each animal, and Blumenthal[8] found that the "assimilation limit;" *i.e.*, the amount which can be injected intravenously without causing glycosuria, is practically invariable for each individual. The velocity of injection plays a part, for Doyon and Dufourt[9] found that more is excreted after rapid than after slow injection. According to them, age, state of nutrition, ligation of the bile duct, and administration of alcohol are apparently without influence. Bang[10] has shown that the glycosuria and glycemia do not run parallel. The concentration of the injected sugar probably plays a part, for Wilenko[11] states that concentrated dextrose solutions (40 per cent) produce the same changes in renal permeability as concentrated salt solutions; that is, first an increased and then a decreased permeability for sugar.

During the injection of dextrose the blood sugar rises and afterwards falls, until it finally assumes a normal or subnormal figure. Von Brasol[5] injected from 0.9 to 5.3 gm. per kilo of body weight in 4 to 6 minutes. Analysis indicated that in the first 2 minutes following the injection some of the sugar had already left the blood; but no direct ratio existed between the amount injected and the blood sugar percentage. After 2 hours the sugar content of the blood was usually normal; with smaller doses (0.9 and 2.3 gm. per kilo) this level was sometimes reached in 1 hour. Butte[12] injected 3 to 10 gm. of dextrose per kilo intravenously and noted the rapid fall of blood sugar to or nearly to the normal level. With 4 gm. per kilo for example, the percentage of sugar in the blood 1¼ hours after the injection was 0.353 per cent, and 2 hours after, 0.220 per cent. In no case did he find a subnormal figure as did Grèhant[13] who found 0.036 per cent blood dextrose 2 hours after an injection of 6 gm. of dextrose per kilo. Pavy[14] found in rabbits that the blood sugar fell rapidly after an intravenous injection; for example, immediately after 4 gm. per kilo had been injected, the blood contained 1.4 per cent sugar; 5 minutes after, 1 per cent; and 15 minutes after, 0.8 per cent. Lépine[15] found that after the injection of 1 gm. of dextrose per kilo slowly into dogs, the blood sugar fell in the course of an hour below 0.1 per cent. Bang[16] injected 1 to 2 gm. per kilo intravenously and found that the maximum blood sugar was reached 2 to 5 minutes after the injection and that the high percentage was maintained for about 30 minutes, after which a rapid fall to normal or below

[7] Gilbert and Carnot, *Compt. rend. Soc. de biol.*, 1898, v, 330.

[8] Blumenthal, F., *Beitr. z. chem. Phys. u. Path.*, 1905, vi, 329.

[9] Doyon, M., and Dufourt, E., *Jour. de physiol. et de path. gên.*, 1901, iii, 703.

[10] Bang, I., Der Blutzucker, Wiesbaden, 1913, 77.

[11] Wilenko, G. G., *Arch. f. exper. Path. u. Pharm.*, 1911, lxvi, 143.

[12] Butte, L., *Compt. rend. Soc. de biol.*, 1896, iii, 274.

[13] Grèhant, quoted by Butte, L., *Compt. rend. Soc. de biol.*, 1896, iii, 274.

[14] Pavy, F. W., *Jour. Physiol.*, 1899, xxiv, 479.

[15] Lépine, R., Le diabète sucré, Paris, 1909, 200.

[16] Bang, I., Der Blutzucker, Wiesbaden, 1913, 74.

took place. Using animals with ligated ureters, Weyert[17] found that after the injection of 4.4 to 5.8 gm. of dextrose per kilo, the blood sugar was down to nearly normal limits in 3 hours, and Harley[18] found that with a dosage of 10 gm. per kilo, the blood sugar fell to normal in from 3 to 6 hours.

Weyert[17] records that the sugar concentration of the lymph runs close to that of the blood. He also found traces of sugar in the cerebrospinal fluid and in the vitreous humor of the eye. None can be found in the saliva except after very large doses,[9, 17, 19] nor is an appreciable amount secreted by the intestine[9, 20, 21] or by the wall of the urinary bladder.[22]

The possibility of the conversion of injected dextrose into glycogen must, of course, be considered. Voit[23] injected subcutaneously 50 gm. of dextrose into three rabbits and found in the liver only 1.4, 2.2, and 7.0 gm. of glycogen, respectively, showing that sugar is not as readily converted into glycogen by the liver when injected parenterally as when fed. Harley[18] after injecting 10 gm. of dextrose per kilo intravenously into dogs with the ureters tied, found some slight evidence of glycogen formation. Freund and Popper[24] analyzed a lobe of liver before injecting dextrose intravenously and the rest of the liver afterwards, and found a small increase in liver glycogen, which was greater if the animals had previously been starved for a short time. With starved dogs 4 gm. per kilo gave no increase in glycogen, but 7 to 11 gm. per kilo resulted in the formation of 1.3 to 7 gm. of glycogen.

Inasmuch as the injected sugar is accounted for only in small part by glycogen and by the various secretions, the blood at the same time rapidly assuming its normal content of sugar, the question naturally arises: What is the fate of the rest of the sugar? In seeking to answer this question, various facts have been brought out. Von Brasol[5] injected 12 to 18 gm. of dextrose per kilo intravenously into rabbits in 30 to 45 minutes and then analyzed the blood, the urine, and mixed samples of muscle, kidney, and liver. Estimating the relation of blood to body weight even as high as 12 per cent and even assuming that all the tissues, including bones, hair, etc., contained the same proportion of dextrose as the tissues analyzed, he calculated that from 17.5 to 28.7 per cent of the sugar administered, was still to be accounted for. Von Brasol did not determine glycogen or other substances; in fact he suggests that the missing fraction has been converted into glycogen, or lactic acid or some other substance. Butte[12] made muscle analyses after injecting intravenously 4 gm. of dextrose per kilo into a dog. Assuming

[17] Weyert, F., *Arch. f. Physiol.*, 1891, 187.
[18] Harley, V., *Arch. f. Physiol.*, 1893, Supplement, 46.
[19] Jappelli, A., *Ztschr. f. Biol.*, 1908, li, 435.
[20] Bang, I., Der Blutzucker, Wiesbaden, 1913, 80.
[21] Kleiner, I. S., *Jour. Exper. Med.*, 1911, xiv, 274.
[22] Kleiner, I. S., *Jour. Exper. Med.*, 1913, xviii, 310.
[23] Voit, C., *Ztschr. f. Biol.*, 1891, xxviii, 245.
[24] Freund, E., and Popper, H., *Biochem. Ztschr.*, 1912, xli, 56.

that the muscle contained no sugar before the injection, he noted a rise to 0.42 per cent half an hour after the injection and then a progressive fall in the percentage of dextrose. This demonstrates, according to Butte, that one part of the injected sugar is transformed in certain organs, although he does not mention the likelihood of bacterial action in a dead animal. In two experiments on rabbits Bang[20] analyzed the liver, skin, blood and lymph, kidneys and urine, muscles, intestines, and bones for free dextrose after injecting 4 gm. intravenously into each animal. The total amount recovered was 47.9 and 75.5 per cent, respectively. But, according to Bang, even these figures are too high because (a) preformed sugar was not determined, (b) the tissues were not blood-free and hence blood sugar was counted twice, and (c) all reduction was not necessarily due to sugar.

The rapid transformation of dextrose into simpler substances, particularly lactic acid, has been suggested by many investigators. Apparently the only one who actually tested for lactic acid after intravenous injections of dextrose was Harley.[18] He found a higher percentage of lactic acid in the blood after dextrose injections into animals with the ureters tied, and also an increase in this substance in the liver and muscle. He also obtained qualitative tests for ethyl alcohol and acetone in the blood. Although some workers have brought forth evidence in favor of a protein origin for lactic acid, there seems to be no doubt that it can be formed from sugar. For example, Embden and his collaborators[25] by perfusing livers with blood found that the liver could form lactic acid from glycogen in the liver or dextrose in the blood. More recently Levene and Meyer[26] have observed a direct conversion of sugar into lactic acid by leukocytes.

An increase in the respiratory quotient after intravenous dextrose injections has been observed by Harley,[27] Verzár and Fejér,[28] and others.

The following points appear, therefore, to be established: (1) The intravenous injection of large quantities of dextrose is followed by an increase in the percentage of sugar in the blood. (2) The high content of sugar in the blood falls rapidly after the end of the injection and assumes the normal value in a comparatively short time, even when the ureters are tied. (3) The kidneys eliminate a considerable, but variable, fraction of the injected sugar. (4) No sugar, or mere traces are to be found in the cerebrospinal fluid, saliva, intestinal secretions, or in the secretion of the mucosa of the urinary bladder. (5) An increase of glycogen in the liver occurs with very large doses, but it accounts for only a small fraction of the sugar injected. (6) Tissue analyses, although few in number, show that some of the sugar is to be found unaltered in various organs. (7) Some of the sugar is oxidized, or at least the respiratory quotient is increased.

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[25] Embden, G., *Centralbl. f. Physiol.*, 1904–05, xviii, 832. Embden, G., and Kraus, F., *Biochem. Ztschr.*, 1912, xlv, 1.

[26] Levene, P. A., and Meyer, G. M., *Jour. Biol. Chem.*, 1912, xi, 361.

[27] Harley, V., *Jour. Physiol.*, 1894, xv, 139.

[28] Verzár, F., and von Fejér, A., *Biochem. Ztschr.*, 1913, liii, 146.

The chief purpose of the present work was to study the disappearance of sugar from the blood under various conditions. In order to obtain a basis of comparison a series of experiments was first carried out in which dextrose was injected into normal animals.

EXPERIMENTAL PART.[29]

The experiments were performed on dogs. Ether was administered by intratracheal insufflation, and cannulas were inserted in the left jugular vein, the right carotid artery, and the neck of the urinary bladder. In a few of the later experiments only morphine and cocaine were employed as anesthetics. In the experiments in which the kidneys were ligated, they were reached through the lumbar region. At the completion of all operative work, the animal was given morphine, usually 0.01 gm. per kilo, subcutaneously, and then the insufflation and ether anesthesia were discontinued. After 1½ to 2¼ hours a sample of blood was taken from the artery, and a warm solution of 20 per cent dextrose was injected slowly into the jugular vein. The rate of injection was about 2.5 to 3 cc. per minute and the dosage was 4 gm. per kilo of body weight. At the middle and end of the injection and at intervals thereafter, samples of blood (from 10 to 30 gm.) were taken for analysis. Urine elimination was divided into periods corresponding to the blood samples taken. During the experiment the animal was kept on an electric thermal pad and at the end of the experiment it was chloroformed.

The blood was analyzed for dextrose by removing the protein by Reid's method,[30] and the reduction was determined by means of a Pavy[31] solution. The urine

[29] A preliminary report of this work was published by Kleiner, I. S., and Meltzer, S. J., *Am. Jour. Physiol.*, 1914, xxxiii, p. xvii.

[30] Reid, E. W., *Jour. Physiol.*, 1896, xx, 316. Vosburgh, C. H., and Richards, A. N., *Am. Jour. Physiol.*, 1903, ix, 35. Macleod, J. J. R., *Jour. Biol. Chem.*, 1908–09, v, 443.

[31] The reagent used was modified from time to time. Vernon (*Jour. Physiol.* 1902, xxviii, 156) suggested the use of twice as much Rochelle salt, potassium hydroxide, and ammonium hydroxide as Pavy originally employed. As this results in the formation of a heavy crystalline deposit in the reagent on standing, we have decreased the amount of Rochelle salt and potassium hydroxide. The resulting solution, which deposits very little precipitate, gives good results. The composition of the solution, as we now prepare it, is:

$CuSO_4$	4.158	gm.
Rochelle salt	14	"
KOH	17	"
NH_4OH (sp. gr. 0.88)	600	cc.
H_2O to	1,000	"

10 cc. of this solution corresponds to 0.005 gm. dextrose, but one should determine the exact equivalent every time the reagent is made up, and this should be checked at intervals.

was also analyzed by the Pavy method. Although this method is not as accurate as some of the other sugar methods, its rapidity, and the fact that a number of determinations can be made from a small quantity of a given solution (as each test requires only 5 mg. of dextrose), led us to use it in this investigation. In some experiments, however, the Bertrand method was used to determine the reducing power of the blood after removal of the proteins, and in a few of the experiments we have used the blood sugar method of Lewis and Benedict as modified by Myers and Bailey,[32] which we have recently adopted for use in this laboratory. With this method only 2 cc. of blood is required.

Normal Animals.

The first dextrose injections were given to seven normal animals. The following is a typical protocol.

Experiment LD 5.—White bull terrier, female; weight 6.5 kilos.

11.00. Etherized. Intratracheal insufflation begun and ether anesthesia continued by this method. Cannulas now inserted in the left jugular vein, left carotid artery, and in the neck of the bladder.

11.51. Urine obtained from bladder; 1.69 per cent dextrose.

11.56. Ether discontinued.

12.02. 6.5 cc. 1 per cent morphine sulphate injected subcutaneously.

12.13. Insufflation discontinued.

2.07. Blood taken, 22.3 gm.; 0.28 per cent dextrose.

2.09. Urine, 77 cc. + (about 5 cc. lost), 8.84 per cent = 6.81 gm. dextrose.

2.10. Injection of warm 20 per cent dextrose solution into jugular vein started.

2.34. Blood taken, 9.5 gm., 0.68 per cent (67 cc. of dextrose have been injected).

2.36. Urine, 40 cc., 6.84 per cent = 2.74 gm. (70 cc. of dextrose have been injected).

2.56. Dextrose injection ends. Total amount injected 130 cc., or 26 gm. in 46 minutes.

2.57. Blood taken, 10.0 gm., 0.93 per cent.

2.58. Urine, 81 cc., 5.50 per cent = 4.46 gm.

3.27. Blood taken, 16.6 gm., 0.40 per cent.

3.28. Urine, 51 cc., 7.32 per cent = 3.73 gm.

3.57. Blood taken, 17.9 gm., 0.30 per cent.

3.58. Urine, 11.5 cc., 10.6 per cent = 1.22 gm.

4.27. Blood taken, 21.3 gm., 0.28 per cent.

4.29. Urine, 3.2 cc., 8.59 per cent = 0.28 gm.

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[32] Lewis, R. C., and Benedict, S. R., *Jour. Biol. Chem.*, 1915, xx, 61. Myers, V. C., and Bailey, C. V., *Post-Graduate*, 1915, xxx, 31; *Jour. Biol. Chem.*, 1916, xxiv, 147.

Summary.—1½ hours after the end of the dextrose injection the blood sugar fell to its original level of 0.28 per cent.

	gm.
Dextrose injected	26.0
Excess dextrose (above original value) in circulating blood at end of experiment	0.00
Dextrose contained in blood samples taken for analysis	0.13
Dextrose in urine	12.43
Dextrose accounted for	12.56
(or 48.5 per cent of the amount injected)	
Dextrose not accounted for	13.44
(or 51.5 per cent of the amount injected)	

The results of the seven experiments are given in Table I.

Considering first the glycemia, it is seen that in five out of seven experiments, the original blood sugar was high. This was to be expected, as the animals had been subjected to being tied down, to anesthesia (ether and morphine), and to operative procedure, all of which are known to raise the blood sugar.[33] An initial rapid rise in the blood sugar during the injection is indicated in each case by the figures for the beginning and middle of the injection. Then a more gradual rise occurred until the end of the injection, after which the percentage of blood sugar fell rapidly for half an hour and then more slowly. At the end of 1½ hours the percentage of sugar in the blood fell to its original level in three experiments (Experiments 3, 5, and 46) and nearly to its original level in two others (Experiments 6 and 7). In the two remaining experiments (4 and 47) the last figures were twice as great as the first. However, in one of these two (Experiment 47) the last percentage found was, nevertheless, a low figure when compared with the rest of the series. In other words, in only one of the seven experiments did the blood sugar fail to fall to what might be termed a low figure for this series.

In Experiments LD 4, 5, and 6, and LP 46, the urine secreted during the injection contained more sugar than that secreted during the 90 minute after-period. This was due to a greater volume of urine being secreted at first rather than a higher percentage of sugar.

[33] For a discussion of these points see Shaffer, P. A., *Jour. Biol. Chem.*, 1914, xix, 297. Loewy, A., and Rosenberg, S., *Biochem. Ztschr.*, 1913, lvi, 114. Hirsch, E., and Reinbach, H., *Ztschr. f. physiol. Chem.*, 1914, xci, 292.

TABLE I.

Intravenous Injection of Dextrose into Normal Dogs.

No. of experiment.	Dextrose injected (4 gm. per kilo).		Blood sugar before injection.	Middle of injection.			End of injection.				½ hr. after injection.				1 hr. after injection.				1½ hrs. after injection.				Total volume of urine.	Total amount of sugar in urine.		Sugar not accounted for by blood and urine. Per cent of total amount injected.
				Blood sugar.	Urine.		Blood sugar.	Urine.			Blood sugar.	Urine.			Blood sugar.	Urine.			Blood sugar.	Urine.						
					Volume.	Dextrose.		Volume.	Dextrose.			Volume.	Dextrose.			Volume.	Dextrose.			Volume.	Dextrose.				Per cent of amount injected.	
	gm.	cc.	per cent	per cent	cc.	per cent	gm.	per cent	cc.	per cent	gm.	per cent	cc.	per cent	gm.	per cent	cc.	per cent	gm.	per cent	cc.	per cent	gm.	cc.	gm.	per cent	per cent
LD3...	29.6	148	0.23	0.85	0			0.98	0			0.63	10.5	3.68	0.39	0.33	1.0	5.5	0.05	0.24	2.7	3.7	0.10	15	0.54	1.8	97.0
LD4...	26.8	134	0.20	0.71	35	6.62	2.32	0.80	81	7.31	5.92	0.45	63	7.70	4.86	0.47	8.3	9.38	0.78	0.41	9.9	9.06	0.90	197	14.78	55.1	39.9
LD5...	26.0	130	0.28	0.68	40	6.84	2.74	0.93	81	5.50	4.46	0.40	51	7.32	3.73	0.30	11.5	10.6	1.22	0.28	3.2	8.59	0.28	187	12.43	47.8	51.5
LD6...	26.0	130	0.19	0.56	32	9.80	3.14	0.72	61	9.80	5.98	0.40	49	9.22	4.52	0.28	13.5	10.9	1.47	0.25	11.5	11.54	1.33	167	16.44	63.2	34.2
LD7...	30.0	150	0.11	0.49				0.50				0.25	121	8.68	10.50	0.18				0.15	50	8.06	4.03	171	14.53	48.4	50.4
LP46*..	34.0	170	0.18					0.70	209	7.42	15.51									0.20	112	10.53	11.79	321	27.30	80.3	19.4
LP47*..	35.6	178	0.11					0.76	112	10.10	11.31									0.25	92	13.32	12.25	204	23.56	66.2	31.4
Average			0.19	0.16†				0.77				0.43†				0.33†				0.25						60.2‡	37.8‡
																										51.8§	46.3§

* Only morphine anesthesia. † Average of five. ‡ Average of six. § Average of seven.

Indeed, the urine of the after-period was usually of a higher concentration, and in Experiment LP 47 this was sufficient to cause a greater excretion of sugar in the after-period than in the injection period.

The small flow of urine in Experiment LD 3 was probably due to over-etherization. Excluding this experiment, the average amount found in the urine was 60.2 per cent of the sugar injected. If we now estimate the excess sugar still circulating in the blood (calculating the blood equal to 7 per cent of the body weight) and add this blood sugar to the urinary sugar, we have an average of 62.2 per cent of the injected amount, leaving 37.8 per cent not accounted for. If we include Experiment LD 3, this average is raised to 46.3 per cent not accounted for.

It thus appears from our own experiments on normal animals that approximately 60.2 per cent of the sugar was excreted by the kidneys, while of the remaining 39.8 per cent only an insignificant fraction (2 per cent on an average) remained in the circulation.

Nephrectomized Animals.

In one of our experiments on normal animals (Experiment LD 3) there occurred, as mentioned above, an almost complete suppression of the kidney function during and after the intravenous infusion of dextrose. Nevertheless, the blood sugar returned to its original value as quickly as in the other experiments. We now performed some experiments upon animals after ligating or removing the kidneys. The results of five experiments are summarized in Table II.

The following is a typical protocol.

Experiment LD 33.—Fox-terrier, male; weight 6.75 kilos.

10.18. Etherized. Intratracheal insufflation begun and ether anesthesia continued by this method. The kidneys were now exposed and ligated and cannulas inserted in left jugular vein and right carotid artery.

11.15. Ether discontinued.

11.26. Lid reflex present.

11.27. Blood taken, 11.6 gm., 0.28 per cent.

11.31. Insufflation discontinued.

11.33. 1 cc. 1 per cent morphine sulphate injected intramuscularly.

12.27. Blood taken, 11.35 gm., 0.27 per cent.

12.28. Injection of warm 20 per cent dextrose solution into jugular vein started.

1.22. Dextrose injection ended. Total amount introduced, 135 cc. or 27 gm. in 54 minutes.

1.23. Blood taken, 11.9 gm., 1.08 per cent.

1.29. 0.5 cc. 1 per cent morphine sulphate injected intramuscularly.

1.54. Blood taken, 11.5 gm., 0.69 per cent.

2.24. Blood taken, 14.9 gm., 0.40 per cent.

2.54. Blood taken, 15.95 gm., 0.32 per cent.

Summary.—1½ hours after the end of the injection the blood sugar fell to 0.32 per cent; *i.e.*, only 0.05 above its original level of 0.27 per cent.

	gm.
Dextrose injected	27.0
Excess dextrose (above original value) in circulating blood at end of experiment	about 0.27
Dextrose contained in blood samples taken for analysis	about 0.17
Total dextrose thus accounted for (or 1.7 per cent of the amount injected)	0.44
Dextrose not accounted for (or 98.3 per cent of the amount injected)	26.56

TABLE II.

Intravenous Injection of Dextrose into Nephrectomized Dogs.

No. of experiment.	Dextrose injected (4 gm. per kilo).		Blood sugar before injection.		Blood sugar at end of injection.	Blood sugar ½ hr. after injection.	Blood sugar 1 hr. after injection.	Blood sugar 1½ hrs. after injection.	Blood sugar 2 hrs. after injection.	Sugar not accounted for. Per cent of total amount injected.
			Before morphine.	After morphine.						
	gm.	cc.	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
LD 32	11.0	55		0.47	1.09	0.93	0.70		0.45	100
LD 33	27.0	135	0.28	0.27	1.08	0.69	0.40	0.32		98.3
LD 34	42.0	210	0.30	0.35	1.0		0.55	0.46	0.29	99.6
LP 48*	43.4	217		0.10	0.62			0.13		99.5
LP 49*	37.0	185		0.28	1.03			0.27		100
Average				0.29	0.96			0.295		99.5

*In these two experiments the kidneys were exposed and prepared for ligation under ether anesthesia, but they were not actually ligated until the effects of the ether had worn off.

In these animals the blood sugar rises to a higher level, but this is not as great as might be expected. In the normal dogs the average figures were 0.19 before injection and 0.77 at the end, a rise of 0.58 per cent; in the nephrectomized animals the corresponding figures are 0.29 and 0.96, a rise of 0.67 per cent; hence there is a difference between the two series of only 0.09 per cent. If we now estimate from this figure how much more sugar there is circulating in the blood in this series, we find that it amounts to less than 1 gm. Therefore during the injection the organism without the kidney has been enabled to rid its blood of nearly as much sugar as if it had its renal function intact. After the discontinuation of the injection the fall in the glycemia is most rapid during the first hour and then becomes slower. In Experiments LD 33, LD 34, LP 48, and LP 49 the blood sugar is either at its original level or only slightly higher at the end of $1\frac{1}{2}$ hours, and in Experiments LD 32 and 34 the figures for 2 hours show that by this time the blood sugar has fallen to or below this value. In Experiment 32 no sample was taken at the end of $1\frac{1}{2}$ hours, but the average for the other four experiments for this time was 0.30 per cent, while the average before injection was 0.25 per cent in the same four.

It is thus evident that after an intravenous injection of dextrose the blood sugar does not rise much higher in nephrectomized animals than in normal animals, and after injection gradually falls to or nearly to its original level. This indicates, of course, that practically all the injected sugar has left the blood stream; and that therefore the body is able to dispose of large amounts of injected dextrose even without the assistance of the kidneys. Furthermore, the fact that this was accomplished in the same length of time as in the normal series, the dosage and other experimental conditions remaining the same, shows that the presence or absence of the kidney has very little influence on the rate of disappearance of dextrose from the blood.

The obvious explanation which offers itself at first thought is that the greater part of the injected sugar is rapidly converted into glycogen by the liver. We have performed some experiments which were designed to exclude this possibility. Dextrose was injected into dogs in which there was practically no circulation posterior to the diaphragm. Animals can easily be prepared in this manner since, with

the aid of intratracheal insufflation, the thorax can be opened and the aorta and vena cava inferior tied near the diaphragm with great facility.

Animals with a Circulation only Anterior to the Diaphragm.

The animals were prepared as follows: An opening was made in the left side of the thorax (under intratracheal insufflation anesthesia) and the aorta and vena cava were ligated as near the diaphragm as possible. The aorta was ligated first, and before ligating the vena cava, pressure was applied to the abdomen in order to increase the amount of blood in the thorax. In some cases Ringer's solution or saline, in others adrenalin, or both, were injected to maintain the blood pressure. These animals, then, had no abdominal circulation; the liver could store no glycogen; the kidneys, pancreas, and adrenals, could not function. Into such animals, we injected dextrose as before and estimated the glycemia at intervals. Some experiments were complicated by such variations as removal of the thyroids, tying the thoracic duct, etc. These additional factors exerted no appreciable influence on the results, and we shall not deal with them in particular. Some of the experiments are summarized in Table III.

A typical experiment of this series is the following:

Experiment LD 30.—Dog; weight 7.5 kilos.

1.40. Etherized. Intratracheal insufflation begun and ether anesthesia continued by this method. Cannulas now inserted in left jugular vein and right carotid artery. Thoracic duct exposed. Opening made in left side of thorax and aorta tied near diaphragm. After pressure on the abdomen the vena cava inferior was tied near diaphragm.

2.43. Wound in thorax closed.

2.44. Ether discontinued.

2.46. Thoracic duct tied.

2.47. Blood taken, 10.9 gm., 0.07 per cent.

2.48–2.50. 40 cc. warm sterile Ringer's solution injected in left jugular vein.

3.04–3.05. 20 cc. Ringer's solution injected.

3.18. 10 cc. Ringer's solution injected.

3.23. Pulse poor. Dog restless. 20 cc. Ringer's solution injected.

3.31–3.32. 20 cc. Ringer's solution injected. (Total quantity Ringer's solution injected, 110 cc. during 44 minutes).

3.40. 1 cc. 1 per cent morphine injected intramuscularly in left fore-leg.

3.49. Blood taken, 10.8 gm., 0.05 per cent.

3.50. Injection of warm 20 per cent dextrose in left jugular vein started.

4.30. Dextrose injection ended. Total amount injected, 150 cc. 20 per cent dextrose or 30 gm., in 40 minutes.

4.32. Blood taken, 14.7 gm., 1.65 per cent.
4.48. Pulse good.
5.27. Blood taken, 22.6 gm., 1.16 per cent.

Summary.—Estimated weight of anterior part of animal; *i.e.*, one half of total body weight..... 3,750 gm.

Quantity of blood circulating (7 per cent of weight).....	262	cc.
Fluid injected.....	260	"
	522	"
Blood samples taken for analysis.....about	39	"
Total circulating fluid.....about	483	"
Excess dextrose circulating ((1.16–0.05 per cent) × 483 cc.)	5.36	gm.
Dextrose in blood samples taken.....	0.26	"
Total dextrose accounted for.....about	5.62	"
Dextrose injected.....	30	"

Amount of dextrose not accounted for, 24.4 gm. or 81 per cent of the quantity injected.

The interpretation of these figures is made difficult by several complications. As we injected the same amount of dextrose (4 gm. per kilo) as in the first two series, the dosage of dextrose per kilo was manifestly higher in these experiments since the posterior part of the body was not fed by the circulation. Exactly how much higher cannot be said definitely, but probably the remaining circulation received about twice as much dextrose per kilo as in the experiments on the entire animal. The infusion of Ringer's solution and the occasional use of adrenalin also make these experiments not entirely comparable with the others. However, the first blood sample was always taken after injection of Ringer's solution or adrenalin, so that a comparable initial glycemia was always ascertained.

In these experiments the blood sugar rose greatly; at the end of the injection it reached from 1.35 to 2.41 per cent, an average of 1.93 per cent for eleven experiments. In considering these figures it must be remembered that, as stated above, the dose of sugar per volume of circulating blood was about twice as high as in the other two series. In every experiment there was a rapid fall in the blood sugar after the injection had been finished. Since the samples of blood were not always taken at the same intervals, it becomes necessary to study the experiments in groups. In Experiments 55, 56, and 57 the

TABLE III.

Intravenous Injection of Dextrose into Dogs with Aorta and Vena Cava Ligated near the Diaphragm.

No. of experiment.	Dextrose injected (4 gm. per kilo).		Blood sugar before injection.		Blood sugar at end of injection.	Blood sugar ½ hr. after injection.	Blood sugar 1 hr. after injection.	Blood sugar 1½ hrs. after injection.	Blood sugar 2 hrs. after injection.	Muscle before injection.		Muscle after injection.		Sugar not accounted for. Per cent of total amount injected.	
										Dextrose.	Polysaccharides.	Dextrose.	Polysaccharides.	By blood.	By blood and muscle.
	gm.	cc.	per cent		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
LD18.....	18.0	90			2.33		1.44							82	
				(3 hrs. later)										87	
LD20.....	25.6	128	0.15	0.11	2.03		1.16								
				(1 hr. later)										80	
LD21* ‡....	28.0	140	0.14	0.04	2.34		1.55							78	
LD28* §....	41.0	205		0.19	2.18		1.58								
				(1 hr. later)										82	
LD30* ‖....	30.0	150	0.07	0.05	1.65		1.16							83	
LD31† ¶....	41.0	205		0.10	1.65		1.18							86 (1 hr.)	
LD35† *‸* ..	27.0	135		0.40	1.81		1.34		1.13					89(2 hrs.)	
					1.75							0.49	0.21	74	
LD45* **...	34.0	170		0.33	1.35	1.02				0.26	0.15	0.36	0.24	91	82
LD55.....	37.0	176		0.15	2.41	1.70				0.40	0.18	0.71	0.50	81	49
LD56*.....	29.8	144		0.17	1.73	1.38		0.82		0.52	0.54	0.80	0.37	91	86
LD57*.....	36.7	183.7													
Average....					1.93††										
					1.83‡‡	1.37									
					2.00§§		1.34								

* Thoracic duct ligated. † Thoracic duct ligated and thyroid glands removed.
‡ 15 cc. of Ringer's solution injected between first and second blood samples.
§ 100 cc. of Ringer's solution and 1 cc. of adrenalin (in small portions) injected before taking first blood sample.
‖ 110 cc. of Ringer's solution (in small portions) injected between first and second blood samples.
¶ 75 cc. of Ringer's solution injected before taking first blood sample.
*** Ether and magnesium sulphate anesthesia. 40 cc. of Ringer's solution injected before taking first blood sample.
** 60 cc. of Ringer's solution and 0.5 cc. of adrenalin injected before dextrose injection.
†† Average of eleven. ‡‡ Average of last three. §§ Average of first seven.

TABLE IV.

Intravenous Injection of Dextrose into Whole Dead Animals.

No. of experiment.	Interval between heart stoppage and injection.	Dextrose injected (4 gm. per kilo).		Blood sugar before injection.	After injection.						Muscle before injection.		Muscle after injection.			Dextrose not accounted for. Per cent of total amount injected.	
					Time.	Blood sugar.	Time.	Blood sugar.	Time.	Blood sugar.	Dextrose.	Polysaccharides.	Time.	Dextrose.	Polysaccharides.	By blood.	By blood and muscle.
	min.	*gm.*	*cc.*	*per cent*	*min.*	*per cent*	*min.*	*per cent*	*min.*	*per cent*	*per cent*	*per cent*	*min.*	*per cent*	*per cent*	*per cent*	*per cent*
LD48.....	3	21	105		9	1.95										56	
LD49.....	21	30	150		30	3.26	73	3.85†			0.54	0.15	83	0.88	0.83	30	0
					46	2.83*											
LD51.....	25	22	110		30	1.54					0.29	0.14	42	0.60	0.21	65	27
LD53.....	14	18	90	0.02 (?)	37	1.67	67	1.48	157	1.30(1.01)‡	0.33	0.21	161	0.45	0.22	71	62
LD54.....	10	32	160	0.23	30	2.18	60	2.03	105	1.93	0.38	0.30	126	0.64	0.60	64	6

* This sample of blood was obtained from the aorta after tying the inferior and superior venæ cavæ.
† This sample of blood was obtained from the portal circulation.
‡ 100 cc. saline were injected in order to get this sample and the figure 1.30 per cent was calculated on the assumption that the saline and blood were intimately mixed.

average percentage of sugar in the blood at the end of the injection was 1.83 per cent, and a half hour later the average was 1.37 per cent—a fall of 0.46 per cent in half an hour. In Experiments 18, 20, 21, 28, 30, 31, and 35, the blood sugar fell from an average of 2.00 per cent at the end of the injection to 1.34 per cent in an hour; in Experiment 57 the fall continued steadily from 1.73 to 1.38 in half an hour, and to 0.82 per cent in $1\frac{1}{2}$ hours. In Experiment 35 a drop in the blood sugar from 1.81 to 1.34 per cent occurred in the first hour after the end of the injection, while during the second hour only a relatively slight decrease took place; namely, to 1.13 per cent.

In every experiment a large percentage of the injected sugar was not accounted for by the sugar present in the circulating blood, from 74 to 91 per cent disappearing from the blood stream during the time of observation. Another way of demonstrating this is by estimating the percentage of sugar which would have been present in the blood if all the injected sugar was still there and comparing this figure with the actual findings, taking into account the volume of blood drawn and of fluid injected. Such estimations indicate that the blood sugar content would be about 7.7 to 7.9 per cent if all the sugar was in the circulating blood. As a matter of fact the highest blood sugar values in all these experiments ranged from 1.35 to 2.41 per cent, or an average of only 1.93 per cent. It is thus evident that the greater part of the injected sugar left the blood rapidly, in fact before the end of the injection. This disappearance of dextrose from the blood stream went on, then, entirely independently of the abdominal viscera. Hence neither the glycogenic activity of the liver nor an excessive consumption of sugar by or in any abdominal organ can account for the greater part of the dextrose which so rapidly leaves the blood after intravenous infusion.

Analysis of Muscle for Carbohydrates.—In a few of these experiments we analyzed skeletal muscles to determine whether sugar had been stored in them. Higher carbohydrates, as well as dextrose were estimated. A few preliminary studies convinced us that the carbohydrate content of normal muscle is not constant. Therefore it was necessary to determine in each experiment the amount of dextrose and higher carbohydrate present in the muscles before and after the injection.

In Experiments 55, 56, and 57 the procedure was as follows: One fore-leg was amputated before the injection of dextrose and immediately placed in the refrigerator. At the end of the experiment the animal was killed, the other fore-leg removed and also placed in the refrigerator. The muscles were dissected off, put through the grinder twice, and extracted with water in the presence of toluene for about 18 hours in the refrigerator. The fluids were then pressed out and the residue again extracted with water for about 2 hours. All fluids obtained were immediately boiled after adding a little acetic acid and filtered through glass wool. The residue, pressing cloth, etc., were thoroughly extracted with boiling water. The combined fluids were then treated with phosphotungstic acid solution while boiling, filtered, neutralized with sodium hydroxide, acidified with acetic acid, and finally concentrated to a definite volume on the water bath. Estimations of the reducing power were made before and after hydrolysis and the results expressed as dextrose. Hydrolysis was effected by boiling under a reflux condenser for 1½ hours in the presence of 1.8 per cent hydrochloric acid.

In all three experiments there was an increase in the total carbohydrates (*i.e.*, the amount found after hydrolysis) after the injection. There was, however, some slight difference with regard to the behavior of the two types of carbohydrates. In Experiments 55 and 56 the increase in dextrose is practically the same as that of the higher carbohydrates; namely, 0.10 per cent for dextrose and 0.09 per cent for higher carbohydrates in Experiment 55; and 0.31 per cent for dextrose and 0.32 per cent for higher carbohydrates in Experiment 56. In both experiments the time was half an hour. In Experiment 57, where the time was 1½ hours, there was an increase of 0.28 per cent in the dextrose and a decrease of 0.17 per cent in the higher carbohydrates. The fact that the higher carbohydrates were found to be increased after 30 minutes and decreased after 90 minutes might indicate that the higher carbohydrates were more readily utilized than the lower ones, or that dextrose before being oxidized by the muscle must be converted into a polysaccharide. Our evidence, however, comes from only three experiments, and consequently we cannot attach much importance to it.

Does this increase in the carbohydrate content of muscle account for all the injected dextrose which disappeared from the circulation? Consideration of the following figures will show that we are not entitled to answer this in the affirmative. If we assume that the muscle tissue of the dog constitutes approximately 43 per cent of

the body weight, as in man,[34] and that half the body is reached by the circulating blood, we can estimate roughly the increase in carbohydrates of the muscles. Such calculations show only comparatively small percentages of the injected dextrose. Thus in Experiment LD 55 the amount not accounted for is reduced from 91 to 82 per cent by taking the muscle carbohydrates into consideration; in Experiment LD 57 the reduction is from 91 to 86 per cent; and in Experiment LD 56 it is somewhat greater, namely, from 81 to 49 per cent. From this it seems evident that the anterior muscles in these experiments did not contain all the missing sugar. On the other hand, it must be admitted that one cannot draw definite conclusions from the muscle analyses, for which great accuracy cannot be claimed. Furthermore it is possible that, in these "anterior" animals, loss of dextrose may occur through the tissue spaces into the posterior part of the body, which we have not included in our estimations. Finally we have not examined other tissues, *e.g.*, the brain, lungs, spinal fluid, bone marrow, etc., where the carbohydrates might also have increased in amount.

Thus, while we cannot claim that the experiments on the muscles in anterior animals throw definite light upon the fate of all the dextrose which disappears from the circulation after an intravenous injection, the fact remains that a notable increase in the carbohydrate content of the muscles was found.

Experiments with Intravenous Injections of Dextrose into Whole Dead Animals.

The foregoing experiments brought up the question whether this passage of sugar from the blood into the tissues is a vital process; this led to a series of experiments in which dextrose was injected into dead animals.

Method.—The dogs were prepared as in the experiments in Tables I and III on normal and anterior animals, except that, under light chloroform insufflation anesthesia, three or four ribs were resected on the left side in order to expose the

[34] Vierordt, H., Anatomische physiologische und physikalische Daten und Tabellen, 3rd edition, Jena, 1906, 44.

heart. Then full chloroform vapor was given until the heart stopped. In some cases one fore-leg was then removed for analysis, all bleeding points being carefully ligated. Continuing the artificial respiration and massaging the heart by hand, we injected the dextrose solution intravenously, much more quickly, however, than in the experiments upon living animals. The rhythmic heart massage was continued for 5 or 10 minutes, and blood samples were taken from the carotid while massaging the heart.

The following is a protocol of one experiment.

Experiment LD 54.—Dog, male; weight 8 kilos.

10.35. Etherized. Intratracheal insufflation begun and chloroform anesthesia produced by this method. Cannulas were inserted in the left jugular vein and right carotid artery. The thorax was opened on the left side, four ribs being resected and the heart exposed. The left fore-leg was then amputated.

12.08. Left fore-leg placed in refrigerator.

12.10. Full chloroform vapor given.

12.20. The fibrillary twitchings of the heart have ceased.

12.23–12.24. Blood taken from the carotid artery, 23.03 gm., 0.23 per cent.

12.30–12.32½. 160 cc. of warm 20 per cent dextrose solution injected into left jugular vein, followed by 3 cc. 0.9 per cent sodium chloride. Heart massaged during the injection.

12.32½–12.36. Massaging of heart continued.

1.02–1.04. Blood taken from carotid, 20.04 gm., 2.18 per cent.

1.31–1.34. Blood taken from carotid, 16.07 gm., 2.03 per cent.

2.17–2.18. Blood taken from carotid, 20.74 gm., 1.93 per cent.

2.39. Right fore-leg removed and put in refrigerator.

Analysis of Muscle of Fore-Legs.

Control muscle: 0.38 per cent before hydrolysis (dextrose).
0.68 per cent after hydrolysis (total carbohydrates),
or 0.30 per cent polysaccharides.

Muscle after dextrose injection: 0.64 per cent before hydrolysis (dextrose).
1.24 per cent after hydrolysis (total carbohydrates),
or 0.60 per cent polysaccharides.

Increase in total carbohydrates, 0.56 per cent.

Summary.—Amount of dextrose injected, 32 gm. 1¾ hours after the end of the injection, the blood sugar was 1.93, or 1.70 per cent above its original value. There was estimated to be about 636 cc. of blood in the blood vessels at the time of taking the last blood sample.

Increase in dextrose in blood (636 × 1.70 per cent) ....10.8 gm.
Dextrose removed in blood samples..... 0.7 "
Total excess dextrose in blood.....11.5 "
or 36 per cent of amount injected.

Therefore 64 per cent not accounted for by blood.

The amount of muscle was estimated to be 3,300 gm.; that is, 43 per cent of (8,000 gm. (body weight)–340 gm. (amputated leg)).

Increase in total carbohydrates in muscle (3,300 × 0.56 per cent)	18.5 gm.
Total excess dextrose in blood	11.5 "
Total dextrose accounted for by blood and muscle	30.0 "

or 94 per cent of the amount injected.

Therefore, 6 per cent not accounted for.

From Table IV, which summarizes the experiments upon whole dead animals, it is seen that the blood sugar content never reached 4.4 to 4.75 per cent, which we estimate would have been the percentage if all the sugar injected had remained in the blood. The highest blood sugar percentage found was 3.85 per cent in the portal blood in Experiment LD 49; the lowest was 1.48 per cent (except the 1.30 per cent of the same experiment, which is not absolutely reliable because of the method used in obtaining this blood sample). The irregularity in the figures of Experiment LD 49 is due to the fact that the various samples were taken from different parts of the circulation. Although one cannot compute the total amount of sugar in the blood in the dead animal experiments with accuracy, it seemed desirable to obtain a basis of comparison with the other series. We therefore give the figures in the column, "Dextrose not accounted for by blood," estimating the blood at 7 per cent of the body weight and using the last blood sugar figure obtained (except in Experiment LD 49 in which the average was used). It is thus seen that from 30 to 71 per cent of the sugar injected was not present in the blood.

In four of these experiments muscle taken before and after the injections was analyzed, and the increase in total carbohydrates indicated that a considerable proportion of injected sugar was to be found in that tissue. In Experiment LD 49 the dextrose content of the muscle rose from 0.54 to 0.88 per cent in 83 minutes, and the higher carbohydrates from 0.15 to 0.83 per cent. This increase in total carbohydrates of the muscle[35] accounted for practically the entire 30 per cent of the injected sugar which was still unaccounted for by the blood. A similar result occurred in Experiment LD 54,

[35] The proportion of muscle in the body was assumed to be 43 per cent of the body weight.

as all but 6 per cent of the injected sugar was accounted for by the blood and muscle analyses together. Here the rise in muscle dextrose was from 0.38 to 0.64 per cent, and in muscle polysaccharides from 0.30 to 0.60 per cent, the time being about 2 hours. In fact this result was the most striking of all, for here the muscle contained about 58 per cent of the injected dextrose. In Experiment LD 51 the amount of sugar still unaccounted for after 42 minutes was reduced to 27 per cent by the muscle carbohydrates, the dextrose increasing from 0.29 to 0.60 per cent, while the higher sugars increased but little; namely, from 0.14 to 0.21 per cent. This was the shortest of the four experiments. In Experiment LD 53 there was very little increase in the dextrose of the muscle, from 0.33 to 0.45 per cent, and none in the polysaccharide content. As this was the longest experiment ($2\frac{2}{3}$ hours), bacterial activity might be thought of as an explanation of the finding of the smallest increase of carbohydrate in the muscle. The increase in the polysaccharide content of the muscles in three of the four experiments is possibly significant, indicating perhaps a condensation of dextrose, after death, within the muscles.

Experiments with Intravenous Injections of Dextrose into the Anterior Parts of Dead Animals.

In Table V are given the results of four experiments upon dead anterior animals. These experiments were similar to the preceding series except that, before causing the heart to stop beating, the aorta and vena cava were ligated near the diaphragm.

In these experiments we again find high blood sugar values, and again we may compare them with the values which we estimated would exist if all the injected sugar had remained within the blood vessels, making due allowance for fluid injected and for blood samples drawn. The calculations give from 7.3 to 7.9 per cent blood sugar. It is seen that the percentages of sugar are actually much lower; namely, 2.24 to 3.42 per cent, showing that a good deal of the sugar injected into these dead stumps left the circulation rapidly. From Experiments 50 and 58 it would seem that this occurred most quickly in the first 30 to 45 minutes and then proceeded more slowly.

In three of the experiments the muscles were analyzed before and after the injection, and it was found that the muscles were richer in

TABLE V.

Intravenous Injection of Dextrose into the Anterior Parts of Dead Animals.

No. of experiment.	Interval between heart stoppage and injection.	Dextrose injected (4 gm. per kilo).		Blood sugar before injection.	After injection.				Muscle before injection.		Muscle after injection.			Dextrose not accounted for. Per cent of total amount injected.	
					Time.	Blood sugar.	Time.	Blood sugar.	Dextrose.	Polysaccharides.	Time.	Dextrose.	Polysaccharides.	By blood.	By blood and muscle.
	min	*gm.*	*cc.*	*per cent*	*min.*	*per cent*	*min.*	*per cent*	*per cent*	*per cent*	*min.*	*per cent*	*per cent*	*per cent*	*per cent*
LD47*.....	2	27.0	135		15	3.27								55	
LD50*.....	42	23.0	115		18	3.42	43	3.07	0.47	0.57	128	1.03	0.57	58	30
					21	3.39	68	2.92†							
LD58.....	0	31.7	156	0.09	30	2.32	70	2.24	0.44	0.39	88	0.74	0.42	73	56
LD59.....	2	36.0	180	0.16			60	2.34	0.52	0.43	81	0.84	0.51	71	52

* Thoracic duct ligated also.

† This sample of blood was obtained from the aorta after tying the inferior and superior venæ cavæ.

dextrose after the injection than before. The percentage of muscle dextrose rose from 0.47 to 1.03, from 0.44 to 0.74, and from 0.52 to 0.84 per cent, respectively. The figures given in the last two columns of the table were obtained on the assumption that the injected sugar reached only half the body, and further that this half contained the same proportions of blood and muscle as the whole body; namely, 7 and 43 per cent, respectively. The first of these two columns shows that a large proportion of the injected sugar had disappeared from the circulation. In Experiment LD 47 about 55 per cent was estimated to have left the blood in 15 minutes. In the other three experiments, each lasting about 70 minutes, the amount of sugar lost from the blood was about 58, 71, and 73 per cent. From the last column of the table we see that even when we deduct the sugar found in the muscle tissue there is still from 30 to 56 per cent not accounted for. In none of these experiments were we able to account for practically all the injected sugar by the muscle and blood analyses, as in two of the experiments on the whole dead animals.

The increase in the higher carbohydrates of the muscle in these experiments is negligible. In Experiment LD 50 there was no increase; in Experiment LD 58 the increase was from 0.39 to 0.42 per cent; and in Experiment LD 59 from 0.43 to 0.51 per cent. These results are in contrast with those found on the entire dead animals (Table IV) in which there was a considerable increase in the muscle polysaccharides in two experiments and a small increase in another. This difference may perhaps be brought into connection with the observation made by Levene and Meyer;[36] namely, that the pulp or juices of various tissues of the dog are able to convert dextrose into a higher carbohydrate if they are activated by spleen juice. In the anterior animal such a mixture of the products of the spleen and tissues by means of an artificial circulation is excluded.

DISCUSSION.

Large amounts of dextrose were injected intravenously into normal dogs. A variable proportion (an average of about 60 per cent) was eliminated by the kidney during the injection and the 90 minutes

[36] Levene, P. A., and Meyer, G. M., *Jour. Biol. Chem.*, 1912, xi, 353.

which followed. By the end of this time the percentage of sugar in the blood had fallen to, or nearly to, the level found before the injection. Consequently about 40 per cent of the injected sugar was not accounted for by the blood and urine analyses. When the same amount of sugar was injected into nephrectomized dogs, the blood sugar, while rising somewhat higher in the course of the experiment, fell to its original level as quickly as in the normal animals. Therefore nearly all the sugar had left the blood in the same length of time without the aid of the kidneys. When dextrose was injected intravenously into animals which had practically no circulation posterior to the diaphragm, the blood sugar did not reach the height which it would have attained if all the sugar had remained in the blood vessels. Since the dosage, however, was based on the weight of the entire animal, and as these dogs had only about half their circulation, the dosage was really about twice as great as in the first two series. For this reason an exact comparison cannot be made. Nevertheless, it was seen that the blood sugar gradually fell and that from 74 to 91 per cent of the injected sugar had left the circulation during the injection and the after-period, which was usually not more than 1 hour. Even in dead animals the blood sugar did not rise to nearly the percentage it would have reached if the injected sugar had remained in the blood vessels. Here, too, the percentage gradually fell, showing that from 30 to 71 per cent had left the blood without any vital forces taking part. In the last series, the dead anterior animals, a similar result occurred; from 55 to 73 per cent of the injected sugar could not be accounted for by the blood.

All these experiments show that the injected sugar disappeared quickly from the circulation. The first question that arises is: Is all this sugar rapidly burned up by the organism? The oxidation of carbohydrate in the animal body is usually tested by determining the respiratory quotient, a rapid rise of which to or near to unity being taken as an indication of sugar combustion. This has not been done by us, but it is well known that after the ingestion or injection of dextrose into the normal animal a rise in the respiratory quotient occurs. There is, however, no reason to assume that a nephrectomized animal will burn dextrose twice as fast as a normal animal. Yet this would have to be the assumption if we were to explain the

results of our normal and nephrectomized series on the basis of a rapid combustion. Tangl,[37] indeed, has found that after nephrectomy the gas exchange is diminished for $3\frac{1}{2}$ to 4 hours, after which it begins to rise slowly. It therefore seems that a nephrectomized animal does not have a greater metabolism than a normal one during our experiments and probably does not oxidize more sugar. Another argument against rapid combustion is the disappearance of large amounts of sugar from the blood of dead animals. Here it can hardly be a question of increased metabolism.

The transformation of dextrose into glycogen in the liver is undoubtedly only a small factor in the disposal of intravenously injected glucose. This is evident from the work of Harley[18] and, more especially, of Freund and Popper.[24] The latter analyzed lobes of the liver before and after dextrose injection, and, when using a dosage like ours, found no increase in the glycogen content. Our experiments upon anterior animals, in which dextrose disappeared from the blood stream when all the abdominal viscera were cut off from the circulation, demonstrate clearly that the glycogenic function of the liver is not of great significance in tracing the fate of intravenously injected dextrose.

Undoubtedly one of the most important factors is the passage of the sugar into the surrounding soft tissues. In two experiments upon the dead entire animal practically all the dextrose not found in the blood was accounted for by the increased amount of sugar in the muscles. In the other experiments upon dead whole animals and dead anterior animals, from 9 to 38 per cent was estimated to be present in the muscles, and in three living anterior animals the estimations for muscle sugar were 5, 9, and 32 per cent of the amount injected. Evidently, a large and variable proportion of the injected dextrose simply passes through the capillaries into the muscle tissue. No doubt, the same process occurs in other parts of the body, more especially in all the soft tissues. The rapid disappearance of sugar from the blood is therefore, to a large extent at least, not difficult to explain.

However, the passage of dextrose into the tissues is not the only cause for its disappearance, for even in the dead animal experiments

[37] Tangl, F., *Biochem. Ztschr.*, 1911, xxxiv, 1.

we could not account for all the injected sugar in any of the anterior experiments or in two of the four experiments upon the dead whole animal. It is possible that some part of the dextrose is converted into higher carbohydrates in certain tissues. In fact some evidence, presented in the foregoing pages, indicates that this does occur in the muscles. In the living anterior experiments an increase in the higher carbohydrates of muscle occurred in two out of the three cases in which the muscle was analyzed. The same result was obtained in the dead whole animals in three out of four tests, while in the dead anterior animals there was little or no formation of polysaccharides. Apparently a condensation does occur in the muscles. But this does not account for any more of the sugar which has disappeared from the blood because it has already been included in our estimations. It may be that such a reaction occurs to the same or to a greater extent in other tissues and, in that way, a large part of the missing fraction would be accounted for.

The fact that in the dead whole animal a considerable condensation to a higher carbohydrate occurred in the muscles, while in the anterior ones very little was evident, suggests that this reaction may be influenced by an intra-abdominal organ. In the living anterior animals, however, a similar polymerization was found. To harmonize this with the above suggestion one would be obliged to assume that the almost insignificant amount of circulation still existing posterior to the diaphragm in living anterior animals suffices to bring from the abdomen the substance required to aid in the condensation. In the dead anterior animals the artificial circulation maintained at times by massaging the heart would not be enough to bring this about.

As other investigators have suggested, it is possible that a part of the injected dextrose is broken down to lower incompletely oxidized compounds. It would therefore not be entirely burned and at the same time, it would not be found as dextrose. We have no evidence to offer on this point.

SUMMARY.

1. As has been found by other investigators, when a large amount of dextrose is injected intravenously into a normal dog it disappears from the circulating blood in about 90 minutes after the end of the

injection. Varying amounts (an average of 60 per cent) are excreted in the urine.

2. Even in nephrectomized animals the same quantity will leave the circulation in the same length of time as in normal animals.

3. This phenomenon seems to be, at least to a great extent, independent of vital processes, since dextrose, after intravenous injection into dead animals, is found to leave the blood rapidly.

4. The phenomenon is independent of the important abdominal organs, for it also occurs in animals (living or dead) in which the aorta and inferior vena cava have been ligated near the diaphragm, thus abolishing most of the circulation posterior to the diaphragm.

5. The fact that a considerable amount of the sugar passes from the circulation into the surrounding tissues was established by finding an increase in the carbohydrates of the muscle tissue. This was done in the case of the living anterior animals and in the whole and anterior dead animals. In most of these experiments there was also evidence of the formation of polysaccharides in the muscle tissue.

THE FUNCTION OF THE KIDNEY WHEN DEPRIVED OF ITS NERVES.*

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PLATES 81 TO 83.

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The question as to what may be the function of a kidney excluded from all nervous influences is not settled at present, in spite of many experimental attempts by various observers. The reason for this lies in the fact that none of the methods employed to solve the question have been free from criticism. That the kidney is plentifully supplied with nerves is well known; but that these have any further function than that of vasomotor control has not been proved. Attempts to demonstrate secretory fibers to the kidney in either the vagus or splanchnic nerves or their branches have given only conflicting evidence.

Removal of all the nerve filaments at the renal hilus by dissection would exclude the kidney from the nervous system, so that its function could be compared with that of a normal organ. But on examining the situation more closely one finds that such method of nerve removal has been found most uncertain in its results.[1] This is due to the fact that the nerve filaments supplying the organ not only lie in a network closely applied to the renal vessels, but also run partly within the walls of these vessels. It has therefore been suggested to use some chemical means of destruction, such as painting with carbolic acid after the dissection. But even under these circumstances one is never absolutely sure that all nerves have been removed. Also, in such thin walled vessels as the renal vein, car-

* Presented in abstract at the meeting of the Federation of American Societies for Experimental Biology, Boston, Dec. 26, 1915.

[1] Cohnheim, J., and Roy, C. S., Untersuchungen über die Circulation in den Nieren, *Virchows Arch. f. Path. Anat.*, 1883, xcii, 443.

bolic acid extensively used may cause a local reaction leading to thrombosis. For these or other reasons, none of the evidence thus far brought forward in regard to the function of such kidneys is convincing.

It has been shown by workers on the methods and possibilities of blood vessel suture that a kidney removed from the body of an experimental animal, and later reimplanted by restoration of the circulation, is able to support life in a presumably normal fashion. With one exception, however, all such investigators have been engaged in demonstrating the possibility of the operation, and have not concerned themselves with the detailed physiological function of the organ.

In the present investigation it was desired to examine in detail the function of a kidney which had been removed from the body and subsequently replaced. By this method it is certain that the organ is entirely outside the sphere of all nervous influences for a time at least, if not permanently. Furthermore, the response of such a kidney to the various functional tests gives at least indirect evidence on the question of secretory innervation.

HISTORICAL.

Asher[2] is one of the most recent investigators to attempt the demonstration of secretory nerves to the kidney. With his coworkers, Pearce and Jost, he has made observations which seem to show that the vagus nerve carries secretory fibers, while the splanchnic carries inhibitory ones; at least so far as the water output of the kidney is concerned. Jungmann and Meyer,[3] after cutting the splanchnic, found an increase of urine and of sodium chloride delivered from the kidney on the same side as the severed nerve. Rhode and Ellinger,[4] however, obtained different results, which led them to believe that the splanchnic has an inhibitory action. The difficulty seems to lie in the fact that in the methods

[2] Asher, L., Die Innervation der Niere, *Deutsch. med. Wchnschr.*, 1915, xli, 1000. Asher, L., and Pearce, R. G., Die sekretorische Innervation der Niere, *Ztschr. f. Biol.*, 1914, lxiii, 83. Jost, W., Die sympathische Innervation der Niere, *Ztschr. f. Biol.*, 1914, lxiv, 441.

[3] Jungmann, P., and Meyer, E., Experimentelle Untersuchungen über die Abhängigkeit der Nierenfunktion vom Nervensystem, *Arch. f. exper. Path. u. Pharm.*, 1913, lxxiii, 49.

[4] Rhode, E., and Ellinger, P., Über die Funktion der Nierennerven, *Zentralbl. f. Physiol.*, 1913–14, xxvii, 12.

employed to elucidate the problem, the circulatory effects of vagus or splanchnic stimulation completely mask other possible effects.

More recently Pearce[5] has employed the method of Barcroft in which the oxygen consumption of the kidney is used as the index of cellular activity, and he has been unable to confirm his previous work done with Asher.

Transplantation or reimplantation of the kidney has been found surgically possible by Carrel, Stich, and a few others.[6] The most recent worker on this subject has been Lobenhoffer. His is the only work which attempts in any detailed way, to study the function of a kidney so treated. He united the severed renal vessels to those of the spleen in dogs, and was successful in ten instances. After removal of the other kidney, the water and salt output, as well as that of lactose and sugar caused by phloridzin, was studied. His results show that such a kidney is able to meet not only the ordinary demands of life, but also the excessive ones set up by the experimental injections. Further details of his work will be discussed later.

Zaaijer[7] has recently reported the survival and complete health of a dog bearing a single kidney, which had been transplanted to the iliac vessels 6 years previously.

Method.

The experiments were carried out on large dogs of both sexes. After examination of the various regions where the kidney might be placed, it was decided not to transplant the organ, but to reunite it to its own severed vessels; for in this way the nearest approach to normal physiological conditions is secured.

Although the left kidney of the dog is somewhat more accessible than the right, and has slightly longer vessels, the renal artery on the left is often bifurcated, or even may leave the aorta as two separate vessels. This reduces the caliber of the arteries and doubles the amount of time spent in sewing. The right side was chosen, therefore, in nearly all instances.

[5] Pearce, R. G., and Carter, E. P., The Influence of the Vagus Nerve on the Gaseous Metabolism of the Kidney, *Am. Jour. Physiol.*, 1915, xxxviii, 350.

[6] Carrel, A., Doppelte Nephrektomie und Reimplantation einer Niere, *Arch. f. klin. Chir.*, 1908-09, lxxxviii, 379. Stich, R., Über Gefäss- und Organtransplantation mittelst Gefässnaht, *Ergebn. d. Chir. u. Orthop.*, 1910, i, 1. Lobenhoffer, W., Funktionsprüfungen an transplantierten Nieren, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1913, xxvi, 197.

[7] Zaaijer, J. H., Dauerresultat einer autoplastischen Nierentransplantation bei einem Hunde, *Beitr. z. klin. Chir.*, 1914, xciii, 223.

Ether was used by the intratracheal method in all the experiments. This method of administration is important, because by its use the respiratory movements can be reduced to a minimum, or even abolished. A motionless field is thus obtained which is of great aid in accurate and speedy sewing.

The animals were prepared for operation by shaving over the abdomen and far down onto the flanks. This area was then made sterile by soap and water, alcohol, ether, and tincture of iodine. A catheter was placed in the bladder, and in the case of male dogs, was carried off to the left side where it remained during the operation. The surgical asepsis was in every respect as strict as possible; gowns and gloves were worn, although during the actual suture of the blood vessels the gloves had to be removed, following which the hands were carefully coated with sterile vaselin.

Transverse Abdominal Incision.—Incision is made transversely across the abdomen just above the level of the umbilicus, and extending from the outer border of the left rectus abdominis across the right side down to the erector spinæ group of muscles (Fig. 1). This is well shown in the illustrations.[8] Failure to prolong the incision far enough towards the posterior wall of the abdominal cavity prevents easy access to the whole kidney region, and makes the suture difficult or impossible.

The abdomen is entered in layers, careful attention being paid to hemostasis. After incision of the skin and subcutaneous tissues, the fascia of the external oblique is picked up on the right, at the outer border of the rectus. It is lifted up, incised, and the incision prolonged well toward the flank. The internal oblique is similarly treated. The rectus is dissected bluntly from its underlying sheath as far as the middle line, when it is cut through. The left rectus is similarly divided. After entering the abdomen through the linea alba, the transversalis can be quickly cut down in either direction (Fig. 2). The wound gapes widely and needs no retraction. All the intestines

[8] This method of entrance to the abdominal cavity is of great aid in the performance of other operations, such as Eck fistula and those involving the biliary passages or adrenal glands. It is illustrated not because the procedure is in any way new, but because I have found that its value is not generally recognized by laboratory investigators.

except the duodenum and descending colon, are then lifted out of the abdomen to the left, where they lie covered by silk handkerchiefs impregnated with liquid vaselin (Fig. 3).

Preparation of Kidney and Renal Vessels for Suture.—After section of the peritoneum about the kidney, it is lifted out and turned toward the middle line of the body where, surrounded with gauze, it is gently held by the assistant. In this position the renal artery comes first to view, and it is carefully cleaned as far as the aorta of all surrounding tissue and nerves. This is done by blunt dissection and by wiping with dry gauze. The kidney is allowed to fall back into place, and the vein is similarly cleaned as far as the vena cava. The ureter is next stripped downward for about 6 or 7 cm. The field is then carefully washed with salt solution, every bleeding point caught and tied, and after drying, the whole is coated with liquid vaselin, including the kidney and its vessels. The two rubber-covered *serrefines* are then placed on the artery, two others on the vein, and the vessels are cut between them. The ureter is cut long. The kidney is then removed from the body and placed on a clean gauze pad. The gloves are now removed.

Vessel Suture.—The ends of the severed vessels are washed quickly with salt solution from a bulb pipette, till every trace of blood is removed. They are then coated with liquid vaselin. The adventitia is removed from the ends of the arteries in the usual way, after which the whole field within the abdomen is covered with vaselined silk handkerchiefs leaving only the stumps of the renal vessels protruding. These handkerchiefs are held in place by brass clips used by stationers, called "O.K. paper fasteners."

The most exacting and important part of the vascular suture lies in placing the three primary guide or tension stitches. This is made much easier by using a suture armed with two needles, one at either end. In this way the suture can always be passed from within the vessel outward. The circumference of the vessel ends must be accurately divided into thirds by these tension sutures.

The artery, being behind the vein, is sewed first, using an over-and-over stitch. This is followed by suture of the vein. The *serrefine* clamps are then removed from the vein and the vessel is allowed to fill under moderate pressure, rolling it a bit between the thumb

and finger. The artery is next freed while held between the thumb and finger so as partially to control the tension during the first few moments of blood flow.[9] After the vascular suture is ended, the ureter is reunited by the invagination method of Van Hook. Following this, the operative field is cleaned of blood, and the peritoneum readjusted by a few interrupted sutures about the periphery of the kidney. Special pains should be taken to see that the kidney is replaced high enough toward the liver so that there is no angulation of the vein.

Closure of the Abdomen.—The next step consists in closing the abdomen after the bowels have been replaced. This is made certain by using first a mattress suture of stout silk placed in the linea alba. After tying this, the severed abdominal muscles can easily be approximated in layers. A continuous suture is used for the transversalis, including at the same time the peritoneum. Mattress stitches are used for the recti, while the obliques are held by interrupted sutures. Closure of the subcutaneous tissue and skin completes the operation. Silk is used throughout the operation, both for ties and sutures.

The dog is placed in a metabolism cage so that the urine can be accurately collected and measured.

The operation is a difficult one, but after some experience it was found possible to restore the circulation in about an hour following its interruption. Forty-three dogs were subjected to operation, of which sixteen survived in suitable condition for further physiological observations.

Examination of the Renal Function.

The operated animals were divided into two series. In the first, the function of the reimplanted kidney was compared with that of the intact one, at periods varying from 2 days to 3 weeks after the primary operation. For this purpose the animal was anesthetized with paraldehyde, each ureter brought out onto the flank through

[9] The method of suture used is that elaborated by Carrel. Carrel, A., La technique opératoire des anestomoses vasculaires et la transplantation des viscères, *Lyon méd.*, 1902, xcviii, 859; Anastomosis and Transplantation of Blood Vessels, *Am. Med.*, 1905, x, 284; The Surgery of Blood Vessels, Etc., *Bull. Johns Hopkins Hosp.*, 1907, xviii, 18.

lumbar incisions, and the urines were compared. This method has been described in a previous communication.[10]

The dogs of the second series were subjected to removal of the unoperated kidney at times varying from 5 to 14 days after the primary operation. The work of their remaining, reimplanted kidney was later examined and compared with that of control dogs, in whom a single nephrectomy had been done.

Series I.

Two typical protocols will suffice to show the results of this series.

June 17, 1915. Dog 66. Weight 10,454 gm. Operation at 10 a.m.; reimplantation of right kidney.

June 18. Has made a good recovery. Blood urea is 0.302 mg. per liter.[11] Excretion of phenolsulphonephthalein given intravenously is 51 per cent in 2 hours.[11]

June 19. Given paraldehyde,[11] 1.7 cc. per kilo of body weight, by stomach tube, followed by 200 cc. of water. Ureters exposed in loins, and cannulæ introduced delivering into test-tubes. Carotid blood pressure recorded on kymograph.

11.10 a.m. Urine appears without delay from each side. Collected for 1 hour.

12.10 p.m. 5.0 gm. of sodium chloride in 20 cc. of water given intravenously. Good diuresis from each side; urine collected for 1 hour.

1.10 p.m. Animal killed. The blood pressure during the experiment varied from 128 to 138 mm. of mercury, except just after the injection of the hypertonic salt solution. The sutured vessels were free from obstruction.

Microscopic examination of the reimplanted kidney showed the capsular spaces wide for the most part, and the glomerular tufts containing a considerable amount

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[10] Quinby, W. C., and Fitz, R., Observations on Renal Function in Acute Experimental Unilateral Nephritis, *Arch. Int. Med.*, 1915, xv, 303.

[11] As was shown in the communication noted above (Quinby and Fitz[10]) the two most dependable tests of renal function are the excretion of phenolsulphonephthalein and the estimation of the blood nitrogen or blood urea. The urease method of Marshall (*Jour. Biol. Chem.*, 1913, xv, 487) was used. By this the normal amount of blood urea of the dog is found to lie between 0.200 and 0.380 or 0.400 mg. per liter. The phenolsulphonephthalein excretion was estimated by the method of Rowntree and Geraghty (*Jour. Pharmacol. and Exper. Therap.*, 1909–10, i, 579). Paraldehyde was used, because in proper dosage it causes neither diuresis nor lowered blood pressure. It must be used fresh, however, because it loses strength on exposure to the air, thus making the dosage uncertain.

of blood. The cells and nuclei of the tubules were well preserved except in a very few areas where a small amount of cellular desquamation had taken place. The blood vessels were everywhere dilated, but no evidence of thrombosis or infarction could be found. A considerable number of hyaline bodies were seen in the collecting tubules, evidently casts. The uninvolved kidney was normal in all respects. Its capsules showed active diuresis, but the blood vessels were not so evidently dilated as were those of the operated organ.

		Amount of urine.	Percentage of NaCl.	Amount of NaCl.*
		cc.		*gm.*
1st hr.				
Before diuresis	Right	5.0	0.90	0.045
	Left	2.5	0.55	0.013
2nd hr.				
After diuresis	Right	60.0	0.95	0.570
	Left	22.0	0.95	0.209

* The chlorides were determined by the Volhard method at first; later by that of McLean and Van Slyke (*Jour. Biol. Chem.*, 1915, xxi, 361).

We see that here the kidney without nerves shows an increased function over the normal one, both before and after diuresis.

June 30, 1915. Dog 67. Weight 11,363 gm. Left kidney reimplanted.

July 12. Has made a good recovery. Urine free from albumin and sugar. Average daily amount, 195 cc. Given paraldehyde, 1.7 cc. per kilo of body weight, followed by 200 cc. of water. Ureters exposed on flanks and cannulated. Blood pressure tracing from carotid.

11.05 a.m. Flow from right kidney begins a little later than from left which started immediately on delivering ureter. Urine collected for 1 hour after flow from each was established.

12.16 p.m. 5.0 gm. of sodium chloride in 20 cc. of water injected intravenously. Marked diuresis.

1.20 p.m. Animal killed. Blood pressure average was 133 mm. of mercury. Renal vessels without trace of clot, though there were a few adhesions about the kidney. A sample of blood drawn just before death showed 0.354 mg. of urea per liter.

Microscopic examination showed that both kidneys were normal in all respects except that in the one previously reimplanted a few mitotic figures could be found in the tubular cells after careful search. These were evidently the sequel of previous cellular degeneration.

		Amount of urine.	Percentage of NaCl.	Amount of NaCl.
		cc.		*gm.*
1st hr.				
Before diuresis	Right	2.5	1.12	0.028
	Left	3.0	0.93	0.028
2nd hr.				
After diuresis	Right	40.0	0.82	0.328
	Left	36.0	0.96	0.345

In this experiment the operated kidney has a function about the same as that of the normal side.

These two protocols fairly represent the findings in this first series which comprised eleven dogs. For a period following operation varying from 10 to 14 days the denervated kidney shows an increased absolute function both for fluid and salt, as compared with the normal kidney. At times this increase is relative as well. This is true of the unstimulated organ, and especially so of the one subjected to the diuretic action of sodium chloride. Beyond this period, however, the balance is regained, so that each organ, operated and intact, divides the labor in very nearly equal parts.

We see here also that absence of the renal nerves abolishes the temporary inhibition of flow so often seen normally after the handling of the ureters necessary for their exposure in the loin. Urine from the denervated side always flowed immediately on section of the ureter; but in some instances the normal side showed an inhibition lasting for as long as 5 minutes. This is analogous to the temporary inhibition occasionally seen on passage of a ureteral catheter in man.

Series II.

The following is a typical protocol.

Nov. 20, 1915. Large male, of Newfoundland type. Weight 15,900 gm. Intratracheal ether and reimplantation of right kidney. Circulation restored after being interrupted for 1 hour.

Nov. 23, 1915. Has made a good recovery; eats well and does not vomit.

Nov. 30, 1915. Has entirely recovered. Wound healed by first intention.

Dec. 4, 1915. Ether. Lateral incision in left flank through which the kidney was removed after ligation of its vessels with silk. Wound closed in layers.

Dec. 7, 1915. Has made an excellent recovery. Passes 320 to 340 cc. of urine daily. Blood urea, 0.456 mg. per liter. Phenolsuphonephthalein, 60 per cent in

2 hours. Urinary sediment shows a rare blood corpuscle; no casts; a few leukocytes. Sugar and albumin absent.

Dec. 11, 1915. Animal well. The 24 hour amount of urine has fallen somewhat and its concentration has increased.

Dec. 14, 1915. Has a rather marked balanitis, and does not urinate until the demand is imperative. After about 48 hours during which no urine was passed, voided a little over 700 cc.

Dec. 18, 1915. Balanitis has responded to irrigation with boric acid and animal is now well. Output of phenolsulphonephthalein, 50 per cent in 1 hour. No albumin or sugar.

Dec. 22, 1915. Intravenous injection of 500 cc. of normal (0.8 per cent) salt solution. Urine withdrawn by catheter at 30 minute intervals showed the following:

	Amount.	NaCl per liter.
	cc.	*gm.*
1st half hr.	83	10.6
2nd " "	62	12.3
3rd " "	48	13.0
4th " "	11	17.2

The urine before diuresis contained 19.10 gm. of sodium chloride per liter. It is thus seen that the kidney responds quickly to diuresis and regains its equilibrium within a normal time limit.

Jan. 31, 1916. Given 2 gm. of lactose intravenously. At the end of 5 hours 1.72 gm. were found in the urine.

Feb. 7, 1916. The 24 hour amount of urine has been measured for 65 days, giving an average of 180 cc. It contains neither albumin nor casts.

Feb. 9, 1916. Blood-urea 0.320 mg. per liter. 61 per cent of phenolsulphonephthalein is excreted in 1 hour. Animal seems to be perfectly normal.

Four other animals of this series were killed after having shown normal kidney function for a month or longer. In each instance the kidney showed microscopic evidences of some hypertrophy of the elements, which usually occurs after unilateral nephrectomy. In two instances the kidney also showed a small depressed scar in the cortex with sclerosis of the normal elements and infiltration by connective tissue. These areas seemed to be the result of small focal necroses caused by interruption of the blood supply during operation. They were never of any considerable size, so that the function of the organ remained uninfluenced.

The results of this second series show that the life of dogs having a single reimplanted kidney is maintained in a normal manner, as estimated by renal functional tests as well as by other more general methods of observation.

DISCUSSION.

The experiments of the first series show that the immediate effect of loss of nerve control over the kidney is a period of overaction. This occurs in all cases, and in the presence of apparent health, as judged by the general condition of the animal, by the normal content of the blood in urea, and by a normal output of phenolsulphonephthalein. This period exists for a varying time, but balance has always been restored by the end of 2 weeks. The kidney recently deprived of its nerves is without vasomotor control; the organ is tense and appreciably enlarged; its vessels are dilated, and following the increase of blood flow there is an increase of function over that of the normal organ. Resumption of tone on the part of the blood vessels brings again normal function.

The results here would seem to be analogous to those vasomotor changes occurring in the splanchnic area after section of the cord. Following this operation there occurs a marked dilatation of the mesenteric vessels, but in a short time vasomotor control is again established. Vascular tone may be resumed through the intervention of other more peripheral nerve ganglia, or the smooth muscle fibers of the vessel wall may possibly regain their tone without such intervention. Also, in the kidney there are ganglion cells, especially in the region of the renal sinus, which may be responsible for the resumption of vasomotor control. Although we know that fibers of the sympathetic type are able to regenerate much more quickly than are those of the peripheral nerves, that they should be able to grow to the renal blood vessels and resume control over them within 2 weeks after section seems improbable. Certainly nerve control by the normal pathways could never have been regained in Zaaijer's dog whose kidney was sutured to the iliac vessels, or in those of Lobenhoffer who used the splenic vessels.

The time variation in regaining normal function is probably to be explained by the greater or less degree of surgical insult in the indi-

vidual case. No kidney showed normal function after being excluded from the circulation for longer than 1 hour and 20 minutes.

The second series of observations indicates that a single kidney which has been removed from the body and subsequently reimplanted, can maintain normal life for apparently indefinite periods. Also such a kidney is able to respond to the excessive demands made on it by the injection of various test substances. My results in this group of experiments fairly coincide with those of Lobenhoffer, except in a few details. He found that the 24 hour amount of urine passed by his dogs varied between 1,500 and 2,000 cc. This is quite unusual. Normal cage dogs in a large number of observations made by us, are found to pass from 200 to 450 or 500 cc. of urine daily, having a specific gravity of about 1.030. Of course this is but a rough average, since all our animals had water continuously at hand, and must have taken varying amounts from day to day. Their diet was of meat. I feel, therefore, that the continuous excretion of such large amounts of urine tends to suggest the absence of complete return to normal conditions.

Again, in the three infusion experiments reported by Lobenhoffer, his animals put out amounts of water and salt which varied widely, though the quantities infused were the same. In the few infusions done by me the resulting outputs were all within 10 or 12 per cent of each other.

The observations on the relative values of the different methods used for testing renal function made by Quinby and Fitz[10] showed that the estimation of the blood nitrogen, or that part of it composing the blood urea, and the output of phenolsulphonephthalein, together form the best means of measuring renal function. Further experience with these tests in clinical work by many observers has confirmed this opinion. I have therefore been content to follow the dogs of this second series by means of these tests, rather than by phloridzin or lactose, as did Lobenhoffer.

The above results, though they throw no direct evidence on any possible secretory function of either the vagus or splanchnic nerves, seem to suggest that if this exists it must play a minor and infrequent part. Under all the conditions produced both by my experiments and by those of Lobenhoffer, the denervated kidney has been

seen to react in an entirely normal manner. One may ask, therefore, if secretory nerves to the kidney are assumed to exist, under what conditions they are manifest; for no lack of such action seems to be demonstrable. Added to the inability of the present observations to show any failure of kidney function which might be ascribed to lack of secretory nerve influence, is the recent work of Cow,[12] who finds in the duodenal mucosa some substance which has a definite diuretic effect on the kidney by means of a hormone action.

It is probable that vasomotor conditions in the kidney, added to the chemical and hormone action of substances contained in the circulating blood, will be found entirely adequate to explain all variations and types of normal renal function.

SUMMARY.

1. By means of vascular suture it is possible to remove the dog's kidney from the body and later to restore it to its former position.

2. Such a kidney is removed from the control of the nervous system, at least for a time.

3. Examination of the function of a kidney so treated shows an initial period of overaction, as compared with that of the normal kidney.

4. This is followed by balanced action.

5. The more recent tests of renal function show that a single, reimplanted kidney is able to maintain normal life indefinitely.

6. The results of these experiments, together with the evidence already at hand, suggest strongly that secretory nerves to the kidney do not exist.

EXPLANATION OF PLATES.

PLATE 81.

FIG. 1. The transverse abdominal incision extending from the outer border of the left rectus muscle well down into the right flank.

PLATE 82.

FIG. 2. The oblique muscles and both recti have been divided and the abdomen is being opened by incision of the transversalis in a direction parallel to its fibers.

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[12] Cow, D., *Jour. Physiol.*, 1914–15, xlix, 441.

PLATE 83.

FIG. 3. The intestines have been withdrawn for the most part from the abdomen, and lie to the left covered by silk handkerchiefs. The kidney, with its vessels and ureter, lies well exposed. Above the kidney is seen a portion of one of the lobes of the liver. At its inner side lie the inferior vena cava, and the pancreas enclosed by a portion of the duodenum.

FIG. 1.

(Quinby: Kidney Deprived of Nerves.)

FIG. 2.

(Quinby: Kidney Deprived of Nerves.)

FIG. 3.

(Quinby: Kidney Deprived of Nerves.)

A METHOD FOR OBTAINING SUSPENSIONS OF LIVING CELLS FROM THE FIXED TISSUES, AND FOR THE PLATING OUT OF INDIVIDUAL CELLS.

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PLATES 84 TO 87.

(Received for publication, January 15, 1916.)

The only cells of the mammalian body which lend themselves as individuals to accurate experimentation *in vitro* while yet alive are the blood cells, the cells of exudates, and the spermatozoa. In saying this we do not overlook the usefulness of tissue cultivation or of experiments with living tissue fragments, of the transplantable tumors for instance. But both means of study involve, not individual cells, but complexes of different cells, which can be standardized only roughly, and which cannot be broken up into their component elements or protected from confusing factors, such, for example, as are introduced by death and autolysis of the central tissue portions. These difficulties have led us to work out a method whereby living tissue cells can be obtained as individuals in suspension, and, if desired, can be plated out in a culture medium (plasma) just as are bacteria. After growth the cells can be liberated again, and again plated successfully.

The method consists, in brief, in the growth of tissue in plasma, according to Carrel and Burrow's modification of Harrison's technique, and the liberation of the new cells by digestion of the clot with trypsin. We had noted that if the serum of a growing tissue culture is replaced with Locke's solution at room temperature the cells of the growing strands that extend out into the medium sometimes contract into spheres, which may be separate or, when growth has been dense, loosely attached, side by side. The general outline of the culture is maintained because the cells are held in place by the fibrin network; and if serum is added and incubation renewed they again put forth processes, and, joining each other, again form strands. The problem

has been to cause the cells to contract and then to liberate them from the fibrin network. This is readily done with trypsin in Locke's solution (Fig. 1); and the resulting suspension can be freed by filtration of all but individual cells.

Method.

We have used the trypsin powders of Merck, Grübler, and Kahlbaum. It is necessary to free them as far as possible from the ammonium sulphate which constitutes the greater part of their bulk. According to Kirchheim,[1] the trypsin of Merck does not contain ammonium sulphate; but we have found it present in as great amount as in the other preparations mentioned. It should be got rid of by Kirchheim's method. The trypsin powder is shaken briefly in absolute alcohol and allowed to stand while the heavy sulphate settles out. The supernatant flocculus is collected on a filter, rapidly washed with ether, dried in the air, and dissolved in Locke's solution (Locke's modification of Ringer's solution, but without sugar). The yield from 2 gm. of the unpurified trypsin is dissolved in 98 cc. of Locke's solution. The cloudy, yellowish fluid is filtered, first through paper, then through a Berkefeld cylinder (N) to sterilize it, and is distributed in test-tubes and kept in the ice box. It loses very slowly its ability to digest and can still be used after 2 months. 3 per cent trypsin digests plasma clots more rapidly and does not harm most cells; but 5 per cent kills cells. Unpurified trypsin powders can be employed but the results are not so good.

The tissue from which cells are to be obtained should be cultivated preferably in plasma diluted with Locke's solution in order that the fibrin network to be digested shall be slight. A mixture of one part of plasma with three of Locke's solution is a medium suitable for most tissues. If there is need for a thick suspension of cells many bits of tissue should be grown. It is convenient to flood them in small Petri dishes with a thin layer of the dilute plasma. After clotting has taken place each dish is sealed to prevent evaporation, and placed in the incubator. A stout cord dipped in hot, sterile paraffin and thrust between the outer and inner rim of the dish, with one end

[1] Kirchheim, L., *Arch. f. exper. Path. u. Pharm.*, 1911, lxvi, 352.

left free, is useful for sealing. A pull on the free end will release the top of the dish.

When growth is established the trypsin solution, warmed to 37°C., is poured on, filling the dish above the plasma, and incubation is continued. In a few minutes some of the tissue fragments are free, and within about an hour the clot has disappeared and there remains a clear fluid containing numerous tissue particles. This is taken up with a pipette, stirred to break up any loose aggregations of cells, diluted with Locke's solution, filtered through sterile gauze, and centrifugalized. The fine, powdery, yellowish gray sediment will consist of discrete cells, nearly all of them alive. They can be washed repeatedly if need be. We prefer for this purpose the "gelatin-Locke's,"—Locke's solution containing $\frac{1}{8}$ per cent of gelatin,—which, as Rous and Turner showed,[2] protects fragile cells against mechanical injury. If the cells are to be plated again in plasma they need not be washed, but after centrifugalization can be suspended in the Locke's solution used to dilute the plasma. Plating is done, as before, in Petri dishes.

Results.

The cells liberated as individuals by trypsin are those which grow out into the medium in strands or a meshwork, or which wander out separately (connective tissue cells, endothelium (?), choroid, sarcoma, and splenic tissue cells). Thus far we have used successfully the tissue of rat and chick embryos, of rat and chicken tumors, and the normal tissue of young rats. Sheets of growing cells (epithelium) are not readily broken up. Whether individual epithelial cells can be liberated in this way is as yet uncertain. But small groups of epithelial cells are obtained, and bits of striated muscle which live for a brief period when plated again.

The individual cells become approximately spherical when in suspension and the nuclei also tend to, though less perfectly. The change in form is especially noteworthy in the case of elements which, when growing in culture, are stellate or of an attenuated spindle shape with an elongated nucleus. When freed, suspended in

[2] Rous, P., and Turner, J. R., *Jour. Exper. Med.*, 1916, xxiii, 219.

serum, and stained, such cells show no trace of the long protoplasmic processes which they had while growing. With Wright's stain certain of them derived from connective tissue and probably of fibroblastic and endothelial origin have a resemblance to the mononuclear series of the blood (Fig. 2). Their cytoplasm is basophilic. Other cells from the same source are three or four times the diameter of any blood element. These morphological features will be taken up in a later paper.

The freed cells, distributed in plasma as separate individuals and incubated, soon put forth processes and assume their original form. Bits of striped muscle from the embryo may round at the ends, thus gaining a leech shape, and put out short processes (Fig. 4). We have not observed them to proliferate. But the spindle-shaped and stellate cells of connective tissue, sarcoma, and the choroid coat of the eye multiply rather rapidly. If the cells are numerous the plate will show at the end of 24 hours a thick mesh- or feltwork consisting of elements once separate which have reached out and joined each other by means of attenuated processes (Fig. 3). The tendency of scattered cells thus to connect with each other again is striking. At the end of 48 hours the number of growing elements is greatly increased, not only by proliferation but by the "waking up" of cells previously spherical. If small masses of cells are present in the culture, as the result of incorrect filtration, growth from them may be almost explosive, each mass resolving itself into elements that radiate in every direction.

The Replating of Cultures.

The limits of the method have not yet been reached. The freed and plated cells can be liberated anew after growth and successfully plated again in fresh plasma. To judge from our results, the process can be repeated indefinitely. Isolated cells of the chick's choroid continue to form pigment after they have been twice liberated with trypsin and twice replated (Fig. 5).

Cells that have been growing in tissue cultures for more than 24 hours when freed and examined in suspension show, as a rule, fat droplets, and corresponding vacuoles when fixed and stained in the

spherical state. Fat droplets have often been noted in tissue cultures and their source is to some extent known.[3] But they are much less prominent in the culture with its extended cells than in the freed, contracted elements. We wish to emphasize the fact that they develop very early, even when growth is taking place in a dilute plasma medium. Only during the first 24, or rarely the first 48 hours, do the cells appear absolutely normal. Later the culture consists for the most part of abnormal elements. This is true also of the freed and plated cells. It follows that replating should be carried out at least every 48 hours.

Technical Difficulties.

The initial cultures must be free from bacteria if the cells are to be replated after their liberation. For the tryptic digestion liberates not only tissue cells but bacterial colonies, and a single one of these latter can by its dispersion ruin all of the new plates. For this reason it is best to cut up the tissue to be grown, in a sterile, glass-sided box, closed with pieces of rubber dam at the ends, through apertures in which the instruments and tissue are introduced, and the hands thrust, encased in sterile, rubber gloves. A small, glass hood with cloth sides will do nearly as well, and it is useful for the replating of cultures. Needless to say a single contamination at any time will ruin a sequence of plates. If the cells are to be used in suspension it is of less importance.

The centrifugalization to bring down tissue cells brings down also fine débris such as bits of cotton, particles of dust, etc., from the fluid. By the time cultures have been twice digested and plated, enough of this will have been collected to mar their appearance, unless special care is taken. Such care consists in the use of well filtered fluids, and centrifuge tubes closed with corks instead of cotton or gauze stoppers. Much time can be saved if the corks are hollowed to fit over the end of the tube, but with a central core to prevent dislodgement (Text-fig. 1). They may be boiled or autoclaved. The central core should be rather short in order that it may remain uncontaminated when the cork is placed on an unsterile surface.

[3] Lambert, R. A., *Jour. Exper. Med.*, 1914, xix, 398.

TEXT-FIG. 1. Centrifuge tube closed with an easily removable cork designed to keep the contents sterile.

SUMMARY.

Individual, living, tissue cells can be obtained in suspension by digesting with trypsin the clot of growing tissue cultures. Under these circumstances the living cells assume a spherical form. When washed and plated in fresh plasma they put out processes and proliferate. After growth in the new plates has occurred the digestion and plating can be repeated. The limits of the method have not yet been reached. We are at work on a number of the problems which it has opened up.

EXPLANATION OF PLATES.

PLATE 84.

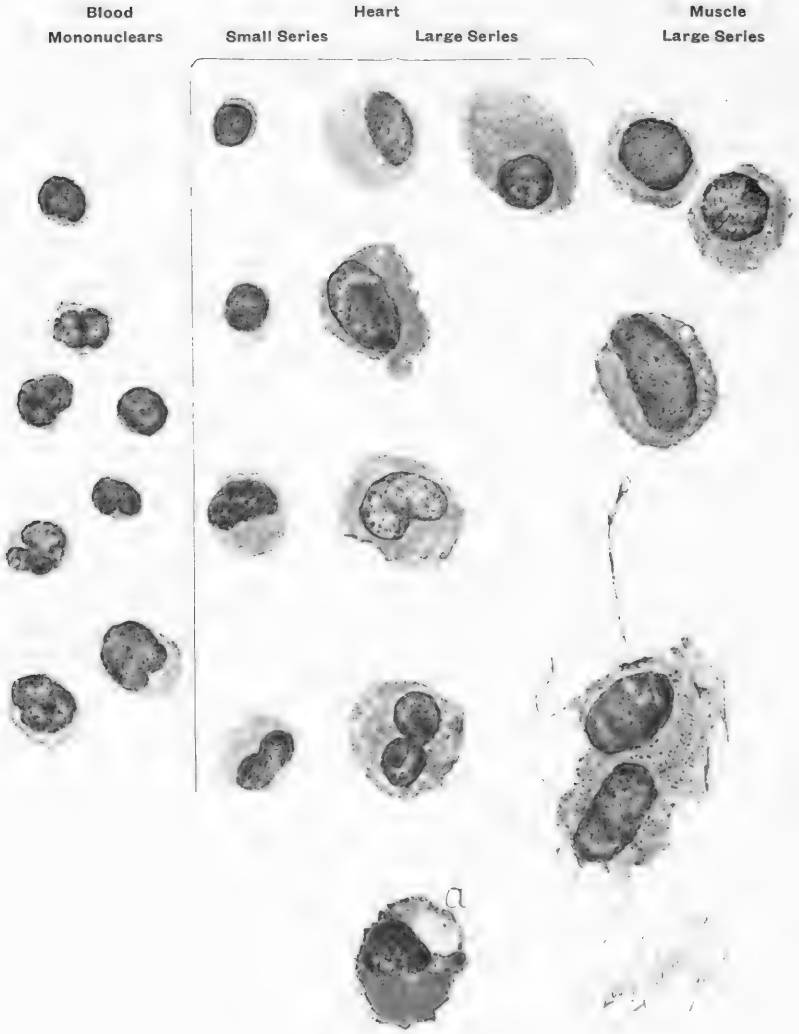
FIG. 1. Edge of a culture undergoing digestion with trypsin. The cells have begun to contract into spheres. (Chick embryo.)

PLATE 85.

FIG. 2. Connective tissue and endothelial (?) cells liberated from cultures of the heart muscle and abdominal muscle of a 3 day old rat. Mononuclear cells from the blood of the same animal. Wright's stain. All the cells are drawn to the same magnification.

FIG. 1.

(Rous and Jones: Living Cells from Fixed Tissues.)



(Rous and Jones: Living Cells from Fixed Tissues.)

FIG. 3

(Rous and Jones: Living Cells from Fixed Tissues.)

FIG. 4.

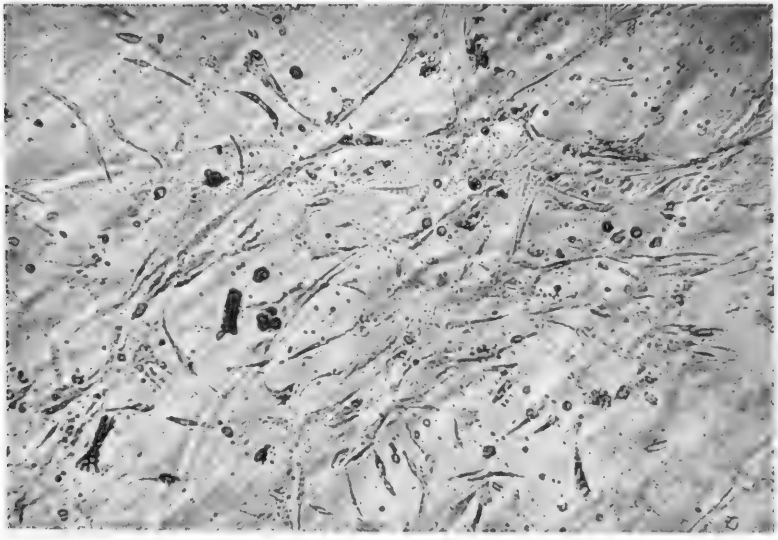


FIG. 5.

(Rous and Jones: Living Cells from Fixed Tissues.)

The cell marked *a* has ingested two red cells. One cell of the muscle series shows vacuoles resulting from a fatty change, and another has attached to it undigested fibrin threads.

PLATE 86.

FIG. 3. Meshwork formed by the anastomosis of connective tissue cells liberated by trypsin and plated as separate individuals. (Chick embryo.)

PLATE 87.

FIG. 4. Striped muscle from a culture incubated 24 hours after liberation by trypsin and replating.

One fragment of muscle, with sharp-cut ends has not grown and has undergone fatty change. But the others give evidence of life, as shown by their change in form, and one has put forth a process. (Rat embryo.)

FIG. 5. Cells from the chick's choroid growing after two liberations with trypsin and two replatings. The formation of pigment is going on actively.

THE PURE CULTIVATION OF SPIROCHÆTA ICTERO-HÆMORRHAGIÆ (INADA).

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Plates 88 and 89.

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Numerous theories exist regarding the etiology of Weil's disease. In June 1914 Inada first observed the occurrence of a spirochæta in this disease, which he designated *Spirochæta icterohæmorrhagiæ*. The spirochæta has since been identified as the etiologic agent of Weil's disease. According to Inada[1] the spirochæta does not grow in solid or semisolid culture media except in a slightly modified medium originally employed by Noguchi for the cultivation of the spirochætæ of relapsing fevers, thus necessitating the use of a piece of fresh tissue as one of the ingredients.[2] The pure culture of this organism produces no odor and is invisible to the naked eye on account of the transparency of its colonies. The spirochætæ grown in these cultures are few in number (Table I), although Inada reports that occasionally there were as many as fifty individuals in a field. Judging from the comparatively poor growth of the spirochætæ, it may be assumed that the method employed by Inada is still far from perfect. Contrary to the experience of Inada, we were able to cultivate the organism in solid or semisolid media, and we have succeeded also in obtaining the culture in a fluid medium.

Material for Cultivation.—Guinea pigs are inoculated intraperitoneally with blood or urine from a person suffering from Weil's disease, and after the animals have developed unmistakable symptoms of the disease, *i.e.*, within 3 to 7 days after inoculation, a sufficient amount of blood is withdrawn, under general anesthesia, from the heart of the animals by means of a sterile syringe, the usual aseptic

[1] Inada, R., *Jour. Exper. Med.*, 1916, xxiii, 377.

[2] Noguchi, H., *Jour. Exper. Med.*, 1912, xvi, 199.

precautions being observed. The samples of blood thus obtained are examined under the dark-field microscope for the spirochætæ and those with a positive finding are selected for the purpose of cultivation. The blood may be used at once or after being kept in a sterile test-tube for several days at room temperature, during which time the spirochætæ increase in number in the majority of instances. The cultures are made in a fluid, a semisolid, or a solid medium.

A small piece or an emulsion of the liver or kidney of animals that have died of experimental Weil's disease may also be used for the purpose of cultivation, but we prefer blood from the heart on account of the comparative ease with which other bacteria can be prevented from entering the culture.

TABLE I.

Date.	No. of spirochætæ in one field.	Date.	No. of spirochætæ in one field.
1915		*1915*	
May 14.....	1– 2	June 15.....	14–15
" 19.....	1– 5	" 18.....	3– 4
" 24.....	1– 8	" 21.....	—
" 26.....	1–18		
June 2.....	1– 4		
" 4.....	4–10		
" 8.....	8–10		
" 12.....	20–30		

Varieties of Culture Media and Mode of Cultivation.

(*1*) *Semisolid and Solid Media.*—Blood agar and blood gelatin were found to be the most satisfactory media. Blood agar is prepared by mixing one part of the blood from a normal guinea pig or a man with one or two parts of the ordinary nutrient agar while the latter is still in a fluid state at a temperature of about 50°C. The blood gelatin is prepared by mixing one part with two to four parts of the blood at a temperature of about 25-30°C., at which the gelatin is still fluid. As soon as the media are prepared, and before they become solidified, the inoculation is made by adding a drop of the infected blood and distributing it well in the media by stirring the tubes. The inoculated tubes are now placed in a temperature any-

where between 15° and 37°C. As a rule, a layer of fluid paraffin about 2 cm. deep is poured into the culture tubes in order to cover the surface, although an abundant growth may be had without the addition of the paraffin oil. It may be remarked that these blood media appear to be a much deeper red as well as opaque near the bottom, as the erythrocytes gradually sediment before the agar or gelatin becomes solidified.

The culture tubes are best left undisturbed for at least 10 to 14 days. The greater the amount of infected blood introduced into the media, the more certain are the chances of obtaining a culture, for the reason that we thereby introduce more spirochætæ. According to our experience blood gelatin gives a better result than blood agar.

The commencement of growth in the solid media is not constant, but this usually takes about 1 week, after which the spirochætæ increase in number for the following 2 to 3 weeks, at which time the growth reaches its maximum. We therefore renew the culture by transplanting it into the new media every week or two. For this purpose the inoculation is made by the stab method.

The growth of the spirochætæ is accompanied by neither an offensive odor nor by the production of a gas and there is no liquefaction of the media.

Morphology.—The spirochætæ grown in our media show active movement under dark-field illumination (Leitz apparatus) and possess a number of small rectangular curves regularly set along the whole length of the organism. It is difficult to count the curves accurately while the spirochætæ are actively moving, but there are some with from two to three curves and some with as many as fifteen curves. The long specimens resemble *Spirochæta pallida*. The body of the spirochæta presents a granular or beaded appearance due to the unequal refractive power as manifested by different sections, the sections with a stronger refractive power being set alternately with those possessing a weaker power. The average organisms show more than fifteen granules and the size of the granules becomes somewhat smaller towards the extremities, which are drawn out into sharp points. In a resting specimen or before motion has begun one of the ends may assume a blunt appearance.

The length of the small specimens attains a quarter of the diameter of an erythrocyte, while that of the long ones is five times as long.

Motility.—The movements are sometimes forward, sometimes backward, and at times certain lateral motions are also noticed. The organism may shoot through the field with great rapidity or display a corkscrew or serpentine movement along the long axis. In a fresh preparation sealed with dammar and kept at room temperature, the spirochætæ become gradually sluggish and finally immobile within varying lengths of time, occasional mobile individuals still being discernible among them.

Number of Spirochætæ.—The number of organisms in a culture may vary according to the age of the latter, but they are generally innumerable, as they gather diffusely or in bundles, and it is impossible to count all the spirochætæ in a field (Fig. 1).

Staining Reaction.—The organisms do not take any of the ordinary aniline dyes, but assume a pinkish or pinkish purple color when stained with Giemsa's solution. The appearance of the stained spirochætæ differs considerably from the morphological features of living specimens (Fig. 2). They are much heavier near the middle and taper off into sharp points at both ends, thus resembling *Spirochæta refringens*. The spirochætæ in a stained preparation are much shorter than the organisms observed under the dark-field microscope. The organisms grow at any temperature between 15° and 37°C., but the optimum temperature is between 20° and 25°C.

Mode of Development.—In a solid culture where the inoculation of the spirochætæ is made in a fluid state the growth is diffuse throughout the media, but in a stab culture they multiply around the stab canal and then spread diffusely. In a young culture the spirochætæ are short and are found near erythrocytes or sometimes attached to them. As time passes the organisms grow longer and wander away from the red corpuscles to form masses or remain scattered. We are unable to decide whether the spirochætæ multiply by longitudinal or transverse division, but we have seen two specimens intertwined. In some instances two spirochætæ are seen to be united at one end, while a bundle of immobile specimens lying parallel may be seen to break up suddenly into units and to move away individually.

(*2*) *Fluid Media.*—Blood serum of man or ox diluted with an equal part of distilled water or undiluted ascitic fluid or pleural exudate is

sterilized by subjecting it to a temperature of 50–60°C. for half an hour for several successive days. Sterile tubes are each filled with 10 cc. of the fluid. Another way of preparing a fluid medium is to follow the method of Noguchi; namely, to add to the above fluid a small piece of kidney from a normal guinea pig and then to use the media after ascertaining the sterility by incubating them for 24 hours. Instead of the kidney, small amounts of coagulum of human or guinea pig blood may be used.

The inoculation of the fluid media is made by introducing one or two loopfuls of the infected blood containing the spirochætæ. The tubes are then placed in a temperature varying from 15–37°C. By this method we have succeeded in obtaining a good growth which was first noticed after 3 to 10 days by the appearance in the clear fluid of a light haze resembling a culture of *Spirochæta pallida*. Upon examination under the microscope numerous spirochætæ identical with those grown in a solid medium were found. Unfortunately, the culture died out in the second generation, probably owing to contamination with a bacillus of the *coli* type or to the lack of the red corpuscles in the fluid media. The transfer in this case was made on the 5th day and the spirochætæ died on the 5th day of the second generation.

A pleural exudate rich in fibrin seems to be the most suitable fluid medium for the purpose of cultivating this organism.

Pathogenicity.—For the purpose of determining the pathogenic property of the pure culture of the spirochæta we have inoculated a small quantity of the culture into the peritoneal cavity of a number of guinea pigs. In the course of 4 to 8 days after the inoculation the animals succumbed after the usual symptoms of the disease. The post-mortem showed all the characteristic lesions. From these animals we have recovered the same organism in pure culture. This experiment completes the links of evidence proving that the spirochæta in question is the causative agent of Weil's disease, and it shows that the pathogenicity of the organism is not noticeably diminished through artificial cultivation. The spirochætæ were found in sections of the liver of the guinea pig from which the culture was derived and of the guinea pig which showed the typical symptoms after receiving the inoculation of the culture (Figs. 3 and 4).

According to Ashizawa's experiment, blood serum from a patient once attacked by Weil's disease has a slight bactericidal action upon the spirochætæ cultivated by our methods.

CONCLUSIONS.

Pure cultures of the spirochætal causative agent of the disease known as Weil's disease, or febrile icterus, in Japan, have been obtained by us in a solid, a semisolid, and a fluid medium. The spirochæta thus isolated remains pathogenic for guinea pigs for many generations. Up to the present time we have succeeded through the courtesy of Professor Nagayo, Dr. Konuma, and Dr. Ishihara, in cultivating three different strains.

The spirochæta is a facultative anaerobe.

The solid and semisolid culture media possess one disadvantage, in that they are opaque on account of the addition of red blood corpuscles; but it is hoped that this drawback may soon be overcome by further studies. We shall report later the results of investigations regarding various questions in immunity as well as further details regarding the biological properties of the spirochæta.

We wish to express our gratitude for the many valuable suggestions and the assistance which Professor Dohi and Dr. Noguchi rendered us during the execution of the present work.

EXPLANATION OF PLATES.

PLATE 88.

FIG. 1. Dark-field view of *Spirochæta icterohæmorrhagiæ* from a pure culture in a semisolid blood gelatin medium. The dark spheroid bodies with a refractive ring are erythrocytes. The white, wavy, beaded lines represent the spirochætæ. Semischematic.

FIG. 2. A film preparation of *Spirochæta icterohæmorrhagiæ* from a pure culture in a semisolid blood gelatin medium. Giemsa's stain. Semischematic.

PLATE 89.

FIG. 3. Distribution of *Spirochæta icterohæmorrhagiæ* in the liver of a guinea pig in which typical symptoms and lesions had been produced by injecting a pure culture of the organism. Levaditi silver impregnation method. × 1,000.

FIG. 4. A film preparation of liver emulsion obtained from a guinea pig which died of experimental Weil's disease produced by a pure culture on the sixth day. Giemsa's stain. × 1,000.

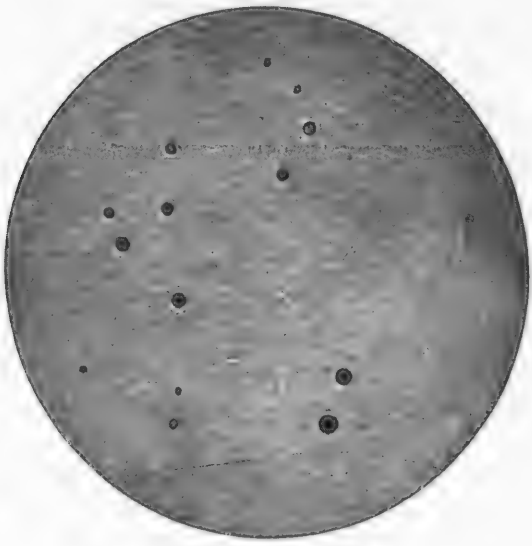
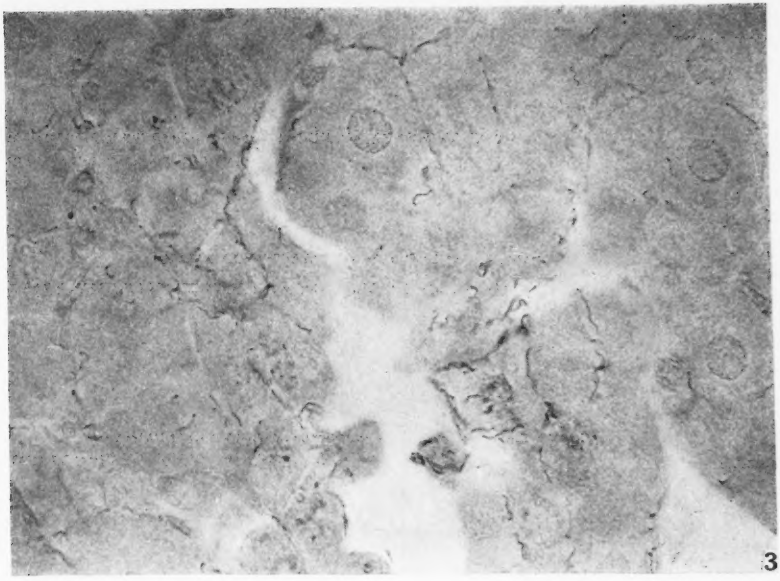
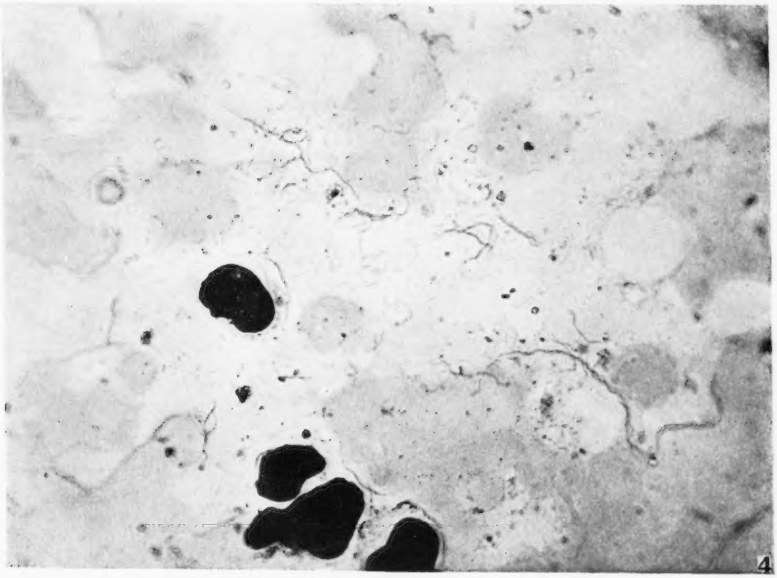


FIG. 1.

FIG. 2.

(Ito and Matsuzaki: Cultivation of *Spirochæta icterohæmorrhagiæ*.)





(Ito and Matsuzaki: Cultivation of *Spirochæta icterohæmorrhagiæ*.)